UA LIBRARIES

MOLECULAR AND MORPHOLOGICAL PERSPECTIVES ON POST-GLACIAL COLONIZATION OF *CLETHRIONOMYS RUTILUS* AND *CLETHRIONOMYS GAPPERI* IN SOUTHEAST ALASKA

By

Amy Marie Runck

RECOMMENDED:

Advisory Committee Chair

Chair, Department of Biology and Wildlife

APPROVED:

Dean, College of Science, Engineering, and Mathematics

Dean of the Graduate School

4-18-01

Date

MOLECULAR AND MORPHOLOGICAL PERSPECTIVES ON POST-GLACIAL COLONIZATION OF *CLETHRIONOMYS RUTILUS AND CLETHRIONOMYS GAPPERI* IN SOUTHEAST ALASKA

A

THESIS

Presented to the Faculty

of the University of Alaska Fairbanks

in Partial Fulfillment of the Requirements

for the Degree of

MASTER OF SCIENCE

By

Amy Marie Runck, B. S.

Fairbanks, Alaska

May 2001

BIOSCI I
QL
737
R666
R86
2001

BIOSCIENCES LIBRARY
UNIVERSITY OF ALASKA FAIRBANKS

I. ABSTRACT

Pleistocene events had a significant impact on the geographic distributions of high latitude organisms. Recently deglaciated, southeast Alaska has been colonized by two species of red-backed voles, *Clethrionomys rutilus* and *C. gapperi*. With distinct biogeographic histories, post-glacial colonization of *C. rutilus* and *C. gapperi* into this region would have occurred by different routes. Variation in the mitochondrial cytochrome *b* gene, the MYH2 nuclear intron, and the post palatal bridge were assessed to examine phylogeographic patterns of these two species, and a proposed contact zone in southeast Alaska. Low, but consistent, levels of sequence divergence of the cytochrome *b* gene were found among four endemic populations, which corresponded with the complex topography of southeast Alaska. Asymmetrical introgression of the mitochondrial genome diagnostic of *C. rutilus* was observed in *C. gapperi*. Post glacial contact resulting from the retreat of the Cordilleran and Laurentide ice sheets has apparently led to the formation of this hybrid zone.

II. TABLE OF CONTENTS

I. Abstract iii

II. Table of Contents iv

III. List of Figures vii

IV. List of Tables ix

V. List of Appendices x

VI. Acknowledgments xi

VII. Introduction 1

VIII. Chapter 1. Morphological and Molecular Analyses of a Post-Glacial Contact Zone in Red-backed Voles (genus *Clethrionomys*).

Abstract 5

Introduction 6

Materials and Methods 9

Mitochondrial cytochrome b restriction fragment analysis 9

Nuclear gene analysis using MYH2 10

Post-palatal bridge analysis 11

Age determination 11

Post-palatal bridge characterization 12

Analysis of the post-palatal bridge 12

Results 13

Mitochondrial cytochrome b restriction fragment analysis 13

Nuclear intron MYH2 13

Post-palatal bridge 14

Concordance of mitochondrial, nuclear, and morphological characters 14

Discussion 15

Post-palatal bridge as a diagnostic character 15

Characterizing the contact zone with three independent markers 16

Origin of the hybrid zone 18

Cytonuclear disequilibrium in the hybrid zone 19

Selection in the hybrid zone 21

Conclusions 22

Acknowledgments 23

Literature Cited 24

IX. Chapter 2. A Phylogeographic Perspective on the Endemic Red-backed Voles of Southeast Alaska (*Clethrionomys rutilus* and *C. gapperi*).

Abstract 50

Introduction 51

Materials and Methods 53

Results 54

Cytochrome b sequences of C. rutilus and C. gapperi 54

Phylogeographic analyses of C. rutilus 55

Phylogeographic analyses of C. gapperi 56

Discussion 57

Geographic variation and colonization in southeast Alaska 57

Subspecific taxonomy and phylogeography of Clethrionomys in southeast Alaska 59

Conclusions 62

Acknowledgments 63

Literature Cited 64

X. Conclusions 88

III. LIST OF FIGURES

Chapter 1. Morphological and Molecular Analyses of a Post-Glacial Contact Zone in Red-backed Voles (genus *Clethrionomys*).

Figure 1. Distribution of *Clethrionomys rutilus* and *C. gapperi* in North America, modified from Corbet (1974) and Hall (1981). 34

Figure 2. Six age categories as determined by second upper molar (M2) root development in *Clethrionomys*. 35

Figure 3. Ossification of the post-palatal bridge. 36

Figure 4. Eight geographic groups created (by latitude) to analyze relationship of age and sex on the post-palatal bridge. 37

Figure 5. Sample sites and distributions of the cytochrome *b* haplotypes of *C. rutilus* (○) and *C. gapperi* (◣) in southeast Alaska and British Columbia. 38

Figure 6. Sample localities and distributions of the nuclear MYH2 sequences in southeast Alaska. 39

Figure 7. Frequencies of complete post-palatal bridges by geographic location. 40

Figure 8. Map of southeast Alaska showing the lack of concordance of the three markers. 41

Chapter 2. A Phylogeographic Perspective on the Endemic Red-backed Voles of Southeast Alaska (*Clethrionomys rutilus* and *C. gapperi*).

Figure 1. Distribution and sampling localities of *Clethrionomys rutilus* and *C. gapperi* and endemic subspecies in southeast Alaska. 70

Figure 2. Maximum parsimony (unweighted) tree constructed from complete cytochrome *b* sequences (1143 bp) for *C. rutilus*. 71

Figure 3. Bootstrap maximum-likelihood using HKY85 + gamma model constructed from complete cytochrome *b* sequences for *C. rutilus*. 72

Figure 4. Maximum parsimony (unweighted) tree constructed from complete cytochrome *b* sequences (1143 bp) for *C. gapperi*. 73

Figure 5. Bootstrap maximum-likelihood using HKY85 + gamma model constructed from complete cytochrome *b* sequences for *C. gapperi*. 74

IV. LIST OF TABLES

Chapter 2. A Phylogeographic Perspective on the Endemic Red-backed Voles of Southeast Alaska (*Clethrionomys rutilus* and *C. gapperi*).

Table 1. Specimens, localities, and voucher numbers. 75

Table 2. Uncorrected pair-wise distances (p) for *Clethrionomys rutilus.* 78

Table 3. Polymorphic cytochrome *b* sites in North American *Clethrionomys rutilus*. 80

Table 4. Uncorrected pair-wise distances (p) for *Clethrionomys gapperi* 81

Table 5. Polymorphic cytochrome *b* sites in Alaskan and Canadian *Clethrionomys gapperi*. 85

V. LIST OF APPENDICES

Chapter 1. Morphological and Molecular Analyses of a Post-Glacial Contact Zone in Red-backed Voles (genus *Clethrionomys*).

Appendix I. Specimens and their localities used in RFLP analysis of cytochrome *b*, and sequence variation of the nuclear MHY2 intron. 42

Appendix II. *Clethrionomys rutilus* and *C. gapperi* specimens that were aged using M^2 root development and scored for completeness of the post-palatal bridge. 46

VI. ACKNOWLEDGMENTS

I would like to thank my family and friends for their support and friendship. I especially thank my parents for their gentle influence on me throughout my life. In addition, I thank my dad for joining me in the field, allowing me to share a part of my life with one of my greatest teachers. My fellow labmates and friends, John Chythlook, Allison Bidlack, Karen Stone, John Demboski, and Chris Conroy were a constant source of intellectual stimulation and great laughs. Cindy Branley kept me grounded in life away from the University.

Special thanks to the folks in the Mammal Collection at the University of Alaska Fairbanks Museum, especially Gordon Jarrell, who always had time to discuss red-backed voles and other Alaskan mammals with me. Kurt Galbreath and Eric Waltari provided many hours of stimulating conversation and good times. Brandy Jacobsen provided excellent help and great conversation in the lab. My exceptionally talented field assistant John Bender II, shared many a long night in southeast Alaska with me, and never faltered. I also thank Steve MacDonald, Leigh Dickey, Howie Chen, Allison Bidlack, and all the prior collecting crews at UAM for their hard work collecting specimens, making this project possible.

I thank my committee, Dr. David R Klein, Dr. Kevin S. Winker for their input and guidance in this project. Lastly, I thank my major professor, Dr. Joseph Cook. His academic and personal guidance throughout this project has been as much as a learning experience as this project. His mentoring in the world of museum collections was invaluable, and I hope I will be able to carry out his philosophy in my future endeavors.

Special thanks to Nella, for extending your home and friendship to me; along with Lucia, Philipe, and Tomás, you were a family away from home.

I thank the USDA Forest Service, US Fish and Wildlife Service, and National Science Foundation for their financial support. The Alaska Cooperative Fish and Wildlife Research Unit and Department of Biology and Wildlife provided endless administrative assistance.

This thesis is dedicated to those special individuals in my life who have helped make this journey so wonderful.

VII. INTRODUCTION

Increased speciation and major range changes of the flora and fauna of North America and Eurasia have been attributed to the climatic fluctuations of the Pleistocene (Hewitt, 1996; Avise et al., 1998; Avise and Walker, 1998; but see also Zink and Slowinski, 1995; Klicka and Zink, 1997). During the height of the ice ages in North America and Europe, distributions of organisms were fragmented into refugia, most of which were found south of the ice sheets. At this time, sea levels were lowered creating Beringia, a high latitude refugium that extended from eastern Asia to western North America.

As ice sheets melted, refugial populations re-colonized recently deglaciated areas (Hewitt, 1996; Hewitt, 1999), and post-glacial contact of some of these genetically divergent populations has resulted in the formation of hybrid zones (Butlin and Hewitt, 1985; Tegelström, 1987; Hewitt, 1996; Wilson and Bernatchez, 1998). The analysis of moderately evolving molecular markers, such as the mitochondrial cytochrome *b* gene, provides insight into the historical processes that shaped contemporary phylogeographic patterns (Avise, 1994; Hewitt, 1999) and provides an opportunity to characterize potential contact zones (Hewitt, 1996; Hewitt, 1999).

The distributions of the northern red-backed vole (*Clethrionomys rutilus*) and southern red-backed vole (*C. gapperi*) in North America have been influenced by geological activities of the Pleistocene. *C. rutilus* is believed to be a recent colonizer of North America, arriving from eastern Beringia during the late Wisconsin glaciation (Gromov and Polyakov, 1977). *C. gapperi*, present in North America since the middle

Pleistocene, persisted in refugia south of the Cordilleran and Laurentide ice sheets during the late Pleistocene (Hibbard et al., 1965; Graham, 1976; Cook et al., in prep). Initially existing in allopatry, these species expanded their ranges into northern Canada as the ice sheets retreated and vegetation colonized previously glaciated areas around 13,500 YBP (MacPherson, 1965; Mann and Hamilton, 1995). Today, *C. rutilus* has a Holarctic distribution, inhabiting northern Europe, Asia, Alaska, and Canada. Restricted to North America, *C. gapperi* encompasses the forests of the Hudsonian and Canadian life zones of central and southern Canada, northern United States, and has populations extending south, into the Rocky and Appalachian mountain ranges (Hall, 1981). These species now share an extensive parapatric contact zone extending from southeast Alaska throughout northern Canada to Hudson Bay.

In Chapter 1, the analyses of three independent markers refined the distribution of *C. rutilus* and *C. gapperi* along their contact zone in southeast Alaska, while testing for hybridization between these two species. In Chapter 2, analyses of sequence variation of the mitochondrial cytochrome *b* gene was used to characterize the genetic relationships, evolutionary and biogeographic histories, and taxonomic status of the five described endemic subspecies (*C. r. glacialis*, *C. g. phaeus*, *C. g. solus*, *C. g. stikinensis*, and *C. g. wrangeli*) in southeast Alaska.

I conducted all of the morphological and molecular work, with the exception of the sequencing of the nuclear intron MYH2, which was done by Brandy Jacobsen. I performed all analyses on these data sets. Dr. Joseph Cook, my advisor and co-author on both chapters, assisted with project design, laboratory facilities, and financial support.

Literature Cited

Avise, J. C. 1994. Molecular Markers, Natural History, and Evolution. Chapman and Hall, London.

Avise, J. C., and D. Walker. 1998. Pleistocene phylogeographic effects on avian populations and the speciation process. Proceedings of the Royal Society of London B, 265:457-463.

Avise, J. C., D. Walker, and G. C. Johns. 1998. Speciation durations and Pleistocene effects on vertebrate phylogeography. Proceedings of the Royal Society of London B, 265:1707-1712.

Butlin, R. K., and G. M. Hewitt. 1985. A hybrid zone between *Chorthippus parallelus parallelus* and *Chorthippus parallelus erythropus* (Orthoptera: Acrididae) I. Morphological and electrophoretic characters. Biological Journal of the Linnean Society, 26:269-285.

Cook, J. A., A. M. Runck, and C. J. Conroy. in preparation. Historical biogeography at the crossroads of the northern continents: molecular phylogenetics of red-backed voles (*Rodentia*: *Arvicolidae*).

Graham, R. W. 1976. Late Wisconsin mammalian faunas and environmental gradient of the eastern United States. Paleobiology, 2:343-350.

Gromov, I. M., and I. Y. Polyakov. 1977. Voles (Microtinae), Fauna of the USSR, Mammals, Volume II, No. 8. Smithsonian Institution Libraries and the National Science Foundation, Washington, D. C.

Hall, E. R. 1981. Mammals of North America. John Wiley and Sons, New York.

Hewitt, G. M. 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. Biological Journal of the Linnean Society, 58:247-276.

Hewitt, G. M. 1999. Post-glacial re-colonization of European biota. Biological Journal of the Linnean Society, 68:87-112.

Hibbard, C. W., C. E. Ray, D. E. Savage, D. W. Taylor, and J. E. Guilday. 1965. Quaternary mammals of North America. Pp.509-525, *in* H. E. Wright, Jr., and D. G. Frey, eds., The Quaternary of the United States. Princeton University Press, Princeton, New Jersey.

Klicka, J. C., and R. M. Zink. 1997. The importance of recent Ice Ages in speciation: A failed paradigm. Science, 277:1666-1669.

Mann, D. H., and T. D. Hamilton. 1995. Late Pleistocene and Holocene paleoenvironments of the north pacific coast. Quaternary Science Review, 14:449-471.

Tegelström, H. 1987. Transfer of mitochondrial DNA from the northern red-backed vole (*Clethrionomys rutilus*) to the bank vole (*C. glareolus*). Journal of Molecular Evolution, 24:218-227.

Wilson, C. C., and L. Bernatchez. 1998. The ghosts of hybrids past: fixation of arctic charr (*Salvelinus alpinus*) mitochondrial DNA in an introgressed population of lake trout (*S. namaycush*). Molecular Ecology, 7:127-132.

Zink, R. M., and J. B. Slowinski. 1995. Evidence from molecular systematics for decreased avian diversification in the Pleistocene epoch. Proceedings of the National Academy of Sciences, 92(13):5832-5835.

VIII. Chapter 1

Morphological and Molecular Analyses of a Post-Glacial Contact Zone in Red-backed Voles (genus *Clethrionomys*) [1]

ABSTRACT

Two red-backed vole species (*Clethrionomys rutilus* and *C. gapperi*), are parapatrically distributed across the boreal taiga of North America. Due to an apparent cline in diagnostic morphological features that converge in areas of contact, the validity of these taxa has been debated. Using three independent markers (RFLP analysis of the cytochrome *b* gene, sequences of the nuclear intron MYH2, and the morphology of the post-palatal bridge), analyses were conducted on 467 individuals from the contact zone in southeast Alaska. Variation in DNA sequence from the nuclear intron and the post-palatal bridge were concordant and indicated that LeConte Bay is the primary physiographic feature dividing these taxa along the North Pacific Coast of North America. Cytochrome *b* haplotypes characteristic of *C. rutilus* were found in *C. gapperi* individuals up to 80 km farther south of LeConte Bay. These results suggest that the mitochondrial genome of *C. rutilus* has introgressed into *C. gapperi* in their zone of contact. Historical phylogeographic evidence suggests that this asymmetric introgression may be the result of post-glacial contact resulting from the retreat of Cordilleran and Laurentide ice sheets approximately 13,500 YBP.

---

[1]Runck, A. M., and J. A. Cook. In prep. Morphological and Molecular Analyses of a Post-Glacial Contact Zone in Red-backed Voles (genus *Clethrionomys*). Journal of Mammalogy.

INTRODUCTION

The late Pleistocene (130,000-10,000 YBP) is believed to have encompassed 23 major climatic oscillations and several minor climate fluctuations (Dansgaard et al., 1993; Greenland Ice-core Project Members, 1993). During glacial advances, ice sheets fragmented northern habitats and changed floral composition, resulting in isolation and modification of geographic distributions of many mammalian populations (Hewitt, 1996; Tremblay and Schoen, 1999; Webb and Bartlein, 1992). Recolonization of recently deglaciated areas by related, but formerly separated, taxa resulted in contact with potential exchange of new alleles in hybrid zones (Barton and Hewitt, 1989; Hewitt, 1996). Additionally, lowered sea levels during full glacial advances resulted in an extensive high latitude refugium, Beringia, that connected Asia and North America and allowed floral and faunal exchanges (mainly from east to west), reshaping species composition in the northern hemisphere.

These Pleistocene events had a significant impact on the distribution of high latitude organisms (Conroy et al., 1999; Heaton et al., 1996; Hewitt, 1996; Klein, 1965). Post-glacial secondary contact has been attributed to the formation of hybrid zones in several taxa, such as red-backed voles (*Clethrionomys glareolus* and *C. rutilus*; Tegelström, 1987), grasshoppers (*Podisma pedestris*; Barton and Hewitt, 1981; *Chorthippus parallelus*; Hewitt, 1993), red-legged partridge and rock partridge (*Alectoris rufa* and *A. graeca*; Randi and Bernard-Laurent, 1999), and mice (*Mus musculus*; Vanlerberghe et al., 1986). Thirty seven percent of contact zones surveyed by Barton and

Hewitt (1985) in North America and Europe (n = 150) were attributed to secondary contact, with the majority apparently arising since the last glaciation.

The distributions of the northern red-backed vole (*Clethrionomys rutilus*) and southern red-backed vole (*C. gapperi*) in North America have been influenced by dynamic geological activities of the Pleistocene. *C. rutilus* is believed to be a recent colonizer of North America, arriving from eastern Beringia during the late Wisconsin glaciation (Gromov and Polyakov, 1977). *C. gapperi*, present in North America since the Middle Pleistocene, persisted in refugia south of the Cordilleran and Laurentide ice sheets during the late Pleistocene (Cook et al., in prep; Graham, 1976; Hibbard et al., 1965). Initially existing in allopatry, these species expanded their ranges into northern Canada as the ice sheets retreated and vegetation colonized previously glaciated areas around 13,500 YBP (MacPherson, 1965; Mann and Hamilton, 1995). Today, *C. rutilus* has a Holarctic distribution, inhabiting northern Europe, Asia, Alaska, and Canada. Restricted to North America, the distribution of *C. gapperi* encompasses the forests of the Hudsonian and Canadian life zones of central and southern Canada, northern United States, and has populations extending south, into the Rocky and Appalachian mountain ranges (Hall, 1981; Figure 1). These species now share an extensive parapatric contact zone extending from southeast Alaska throughout northern Canada to Hudson Bay.

Morphologic similarity of *C. rutilus* and *C. gapperi*, particularly within their zone of contact, has resulted in confusion concerning the validity and relationship of these taxa (Bee and Hall, 1956; Youngman, 1975). Characters that are diagnostic outside of their common boundary, such as the post-palatal bridge and pelage color, appear to vary

clinally and converge in the contact zone (Bee and Hall, 1956; Hall, 1981). Several studies have attempted to clarify the relationship of these two species, but yielded ambiguous results. For example, three studies of bacular morphology of five species of *Clethrionomys* found minimally diagnostic differences between *C. rutilus* and *C. gapperi* (Anderson, 1960; Hamilton, 1946; Hooper and Hart, 1962). Similarly, G-banded chromosomes of *C. rutilus* and *C. gapperi* are nearly identical, with only slight differences existing in the Y chromosome (Nadler et al., 1976; Rausch and Rausch, 1975). Canham and Cameron (1972) examined allozyme variation along the contact zone in the Northwest Territories and British Columbia, but they were unable to consistently identify the two species because the frequency of the α-globulins converged in an areas of contact.

Sequence divergence of the cytochrome *b* gene between *C. rutilus* and *C. gapperi* exceeds 7% (Cook et al., 2001; Cook et al., in prep; Runck and Cook, in prep). Phylogenetic analyses of nine species of red-backed voles indicated that *C. gapperi* and *C. rutilus* are not sister taxa (Cook et al., in prep). This level of sequence variation and the fact that they are not sister taxa support their specific status. However, individuals identified as *C. gapperi* near the Stikine River possessed the cytochrome *b* gene sequence of *C. rutilus* (Cook et al., 2001).

The purpose of this study was to further assess the relationship of these two species, focusing on individuals from the contact zone in southeast Alaska. Because their biogeographic histories suggest post-Pleistocene colonization of the region, these two species provide an opportunity to examine the dynamics of species interactions in a

recently deglaciated contact zone. Variation of three independent markers: mitochondrial DNA cytochrome *b*, nuclear DNA intron MYH2, and post-palatal bridge morphology were analyzed.

MATERIALS AND METHODS

Mitochondrial cytochrome b restriction fragment analysis

Restriction fragment length polymorphism (RFLP) using enzyme digestion was employed to determine the distribution of cytochrome *b* gene haplotypes of *C. rutilus* and *C. gapperi* in southeast Alaska. Sampling focused on the contact zone of the two species in southeast Alaska from which 414 individuals of *C. rutilus* and *C. gapperi* (Appendix I) were analyzed. In addition, 61 *C. gapperi* and 12 *C. rutilus* had been sequenced from southeast Alaska (Runck and Cook, in prep.) and were included in the assessment of haplotype distribution.

Cryogenically preserved tissues (heart, kidney, or liver) were obtained from the of Alaska Frozen Tissue Collection of the University of Alaska Museum. DNA was extracted following a modified salt extraction protocol of Miller et al. (1988).

The polymerase chain reaction (PCR) was used to amplify a 632 base pair (bp) fragment of the cytochrome *b* gene with the primers MVZ 05 (Smith and Patton, 1993) and CLETH 06 (5'CCTGTTGGGTTGTTGGATCCTG 3'). Concentrations and volumes of reagents were 2.5µl of each primer (10 mmol); 0.50 µl dNTPs (10 mmol); 2.5 µl 10X buffer with 1.5 $MgCl_2$; 4.4 µl H_20; 0.125 µl Perkin-Elmer AmpliTaq DNA polymerase; and 12.5 µl DNA template for a total volume of 25.0 µl. Amplification on a Perkin-Elmer 9600 thermocycler followed standard protocols (Lessa and Cook, 1998) and

included negative controls. PCR products were visualized on 1.5% agarose gel stained with ethidium bromide.

Restriction enzyme screening was conducted using the restriction enzyme ALU I. ALU I cut the restriction site AGCT, which was determined to be present once in *C. rutilus* but not in *C. gapperi* by using the computer program DNA Strider 1.2 (C. Mark, Cedex, France) on 73 sequenced cytochrome *b* genes from *C. rutilus* and *C. gapperi*. The amplified cytochrome *b* fragments were digested using 0.35 µl of the restriction endonuclease ALU I, 1.0 µl Buffer2 (New England Biolabs), and 5.0 µl PCR product. Positive controls were included to confirm enzymatic activity in each trial. PCR products were digested for 3 hours at 37°C and then visualized on a 1.5% agarose gel stained with ethidium bromide. Digestion of cytochrome *b* fragments resulted in two bands for *C. rutilus* and in one band for *C. gapperi*.

Nuclear gene analysis using MYH2

The nuclear intron MHY2 (Lyons et al., 1997) was sequenced for 14 *C. gapperi* and 8 *C. rutilus*. Sampling focused on southeast Alaska, but one individual each of *C. rutilus* from Russia, Finland, and interior Alaska, and one individual each of *C. gapperi* from Washington and Minnesota, were included (Appendix I).

The MYH2 locus was amplified using the primer set MYH2F and MHY2R (Lyons et al., 1997) using 5.0 µl of each primer (10 µM); 2.5 µl dNTPs (1 mM); 5.0 µl 10X buffer; 27.25 µl H_20; 0.25 µl Perkin-Elmer AmpliTaq DNA polymerase; 4.0 µl of $MgCl_2$ (25.0 mM); and 1.0 µl of DNA template. Thermocycling was completed on a Perkin-Elmer 9600 thermocycler following standard protocols (Lyons et al., 1997). PCR

products were visualized on a 1.5% agarose gel stained with ethidium bromide. Purification of PCR products was done with Qiagen Dneasy purification kits. Automated sequencing was completed on an Applied Biosystems ®373 automated DNA sequencer at the University of Alaska Fairbanks Core Facility for Nucleic Acid Research.

Sequences were aligned by eye with Sequence Navigator®, Version 1.01 (Applied Biosystems, Inc., version 1.01). Maximum parsimony in PAUP*4.0b4a (Swofford, 2000) was used to assess phylogenetic relationships.

Post-palatal bridge analysis

A total of 301 individuals of *C. gapperi* and 172 *C. rutilus* were analyzed for completeness of the post-palatal bridge (Appendix II). Sampling focused on the contact zone in southeast Alaska but included 31*C. gapperi* from Washington State and 65 *C. rutilus* from interior Alaska.

Age determination

Because age may be associated with closure of the post-palatal bridge (Bee and Hall, 1956), all individuals were aged by the degree of root development in the second upper molar (M^2; Lowe, 1971; Mihok, 1980; Tupikova et al., 1968). Cleaned skulls had their right maxillaries chipped away to expose the entire M^2 while allowing the molar to remain embedded. A Wild microscope (model M5A) was used to characterize M^2 root development into six classes (Martell and Fuller, 1978): anterior groove open; anterior groove closing; anterior groove closed; anterior groove closed with neck formed, roots <1 mm; roots > 1 mm in over-wintered voles (Figure 2).

Post-palatal bridge characterization

Cleaned skulls were examined under 25x magnification to score the post-palatal bridge as either complete, with medial shelf connected to lateral parts of the palate, or incomplete, with medial shelf disconnected (Figure 3). Individuals that appeared to have their bridges altered by dermestid beetle cleaning (n = 214) were not included in this analysis.

Analysis of the post-palatal bridge

Individuals were pooled into eight geographic groups by latitude. Pooling populations increased sample sizes so that analyses related to age classes would be more evenly represented throughout southeast Alaska. The four groups of *C. rutilus* were (1) Denali National Park and Fairbanks, (2) Chilkat Peninsula, Klukwan, Laughton Glacier, and Taiya River, (3) Antler River, Echo Cove, Juneau, Limestone Inlet, Lynn Canal, Powers Creek and Turner Creek, (4) Cape Fanshaw and Patterson River. Four groups of *C. gapperi* included the localities (5) Farm Island, Hut Point, Mallard Slough, Tyee, Unuk River, and Chickamin River, (6) Etolin Island, Wrangell Island, Frosty Bay, Grant Creek, Hoya Creek, and Reflection Lake, (7) Bond Bay, Skull Creek, Foggy Bay, Gwent Cove, Revillagigedo Island, Ledge Point, Rudyerd Bay, Smeaton Bay, Port Stewart, and Union Bay, and (8) Washington State (Figure 4). Six of these geographic groups were in southeast Alaska and each spanned a latitudinal distance of 80-115 km.

To assess variation in the post-palatal bridge formation with age and sex, analysis of variance (ANOVA) was done using SAS (SAS Institute Inc., 1996) with the GLM procedure. Analyses were completed based on the eight geographic groups, and there

was not a significant relationship between sex and ossification of the post-palatal bridge ($p > 0.05$). Because only one of the eight groups possessed a significant relationship with age and post-palatal bridge ossification ($p<0.05$, $R^2=0.25$), and the R^2 value was relatively low, all age groups and both sexes were pooled for additional analyses.

Frequencies of complete post-palatal bridges were computed for each geographic group and plotted with respect to distance between groups.

RESULTS

Mitochondrial cytochrome b restriction fragment analysis

The distributions of the two haplotypes in southeast Alaska (Figure 5) indicate that the cytochrome *b* sequence characteristic of *C. rutilus* was found in all of the individuals of *Clethrionomys* from interior Alaska to the Stikine River. The *C. rutilus* cytochrome *b* gene sequence was also found in individuals south of the Stikine River, extending 80 km south to Walker Cove on the southeast Alaska mainland. These individuals have been identified as *C. gapperi* based on their distributions and morphology (Hall, 1981). The cytochrome *b* haplotype of *C. gapperi* was found on Etolin, Wrangell, and Revillagigedo islands, Cleveland Peninsula, and on the mainland south of Walker Cove (Figure 5). The two haplotypes characteristic of each species had parapatric distributions, but were found in sympatry (on same trap lines) at Walker Cove and Tyee.

Nuclear intron MYH2

This diagnostic sequence had four consistent base pair differences between the *C. gapperi* and *C. rutilus*. The MYH2 sequences of individuals of *Clethrionomys* north of

Mallard Slough in southeast Alaska were identical to *C. rutilus* from Interior Alaska, Finland, and Russia. All individuals from Mallard Slough and south had identical sequences to *C. gapperi* from Minnesota (Figure 6). Sequence divergence between the species was 2.3%.

Post-palatal bridge

To assess clinal variation, individuals were compared across increments of 5° of latitude. The frequency of complete post-palatal bridges in *C. gapperi* individuals from Washington and southeast Alaska was not significantly different, nor was there a significant difference in *C. rutilus* from interior to southeast Alaska (Figure 7). There was, however, a distinct break at the Stikine River area, with individuals north of Mallard Slough having incomplete post-palatal bridges, and individuals from Mallard Slough and south of the Stikine River possessing complete bridges.

Concordance of mitochondrial, nuclear, and morphological characters

The distributions of the mitochondrial cytochrome *b* haplotypes were not concordant with the distribution of the diagnostic morphologic character or nuclear MHY2 sequence (Figure 8). All individuals north of Mallard Slough (n=38) had the post- palatal bridge morph, nuclear sequence, and cytochrome *b* haplotype of *C. rutilus*. Individuals south of Mallard Slough to Walker Cove (n=88) and from the Iskut (n=18) and Stikine rivers (n=12) in British Columbia possessed the post-palatal bridge morph and nuclear sequence of *C. gapperi,* but had the cytochrome *b* haplotype of *C. rutilus*. Specimens from Etolin (n=55), Wrangell (n=55), and Revillagigedo (n=20) islands,

Cleveland Peninsula (n=131), and south of Walker Cove possessed (n=70) the post-palatal bridge morph, nuclear sequence, and cytochrome *b* sequence of *C. gapperi*.

DISCUSSION

Traditionally, morphological markers such as color patterns and measurements have been used to diagnose hybrid intermediates in contact zones (Hewitt, 1988). With the advance of molecular techniques, molecular markers can help elucidate relationships of hybridizing taxa (Avise, 1994). Using both methods can provide additional insight into evolutionary processes that may not be detected by morphological or molecular analyses alone (Bell, 1996; Sattler and Braun, 2000).

Because these two species meet along a north-south distribution pattern, it has been difficult to determine if convergence of diagnostic characters in the contact zone is a result of clinal geographical variation or some level of interspecific hybridization (Bee and Hall, 1956; Canham and Cameron, 1972). The relationship of *C. rutilus* and *C. gapperi* in their zone of contact has previously been examined with either morphological or molecular markers. Therefore, analyses of a diagnostic morphological character (post-palatal bridge) and two independent molecular markers, and their phylogeographic history may provide additional insight into the biological processes that govern the relationship of these two species.

Post-palatal bridge as a diagnostic character

The abrupt change in frequency of complete post-palatal bridges at the Stikine River area does not reflect a clinal pattern, a result that contrasts with the suggestion that variation in the ossification of the post-palatal bridge is a result of a latitudinal cline (Bee

and Hall, 1956). Lack of clinal variation indicates that the degree of ossification is a reliable diagnostic character for identifying the two species. Change in frequency of ossification of post-palatal bridges near the Stikine River coincides with Hall's (1981) distribution and depiction of parapatry of *C. rutilus* and *C. gapperi*.

Characterizing the contact zone with three independent markers

The nuclear MHY2 sequence and post-palatal bridge morphology diagnosed *Clethrionomys* in the contact zone south of the Stikine River as *C. gapperi*. Lack of concordance between the mitochondrial cytochrome *b* gene and these two other markers is a result of either incomplete lineage sorting or genetic introgression of the mitochondrial genome. Incomplete lineage sorting can result in ancestral polymorphisms being retained in some populations (and species) but not others, causing paraphyly (Avise et al., 1984). If lineage sorting has occurred, then only some populations of *C. gapperi* (populations in the contact zone) and all individuals of *C. rutilus* have retained mitochondrial haplotypes belonging to their common ancestor. However, with lineage sorting, it is expected that sequences in common among taxa exhibit levels of divergence proportional to the length of time since separation from their common ancestor. The level of cytochrome *b* divergence between *C. gapperi* and *C. rutilus* is relatively high (8%; Cook et al., 2001), suggesting that they have been separated from their common ancestor for a considerable period of time. If lineage sorting has occurred, relatively high levels of sequence divergence would exist between the shared ancestral haplotypes. However, minimal levels of genetic divergence existed between *C. gapperi* in the contact zone and *C. rutilus* ($p=0.006$; Runck and Cook, in prep.), a level of divergence inconsistent with a

lineage sorting hypothesis for the paraphyly of *C. gapperi*. Lack of concordance among these three markers is best explained as a result of asymmetric introgression of the mitochondrial genome of *C. rutilus* into *C. gapperi*.

Introgression has previously been observed in other *Clethrionomys* species pairs. In a mitochondrial RFLP analysis and protein electrophoretic study, Tegelström (1987) documented the mitochondrial genome of *C. rutilus* in specimens of *C. glareolus* of northern Fennoscandia. Mitochondrial genetic distances between hybrid *glareolus* and *rutilus* were low (p=0.0012 – 0.00129), and several haplotypes existed, suggesting that they had not gone through divergence and lineage sorting (Tegelström, 1987). Hybridization was hypothesized to be a result of secondary contact of the two species after the retreat of the Weichselian ice sheet around 8,000-13,000 ybp.

Eurasian *C. rutilus* and *C. glareolus* produce hybrids (Zimmerman, 1965), and Grant (1974) demonstrated that *C. gapperi* from Quebec and *C. rutilus* from Great Britain could produce fertile offspring, although reduced F1 viability was observed. Though species of *Clethrionomys* are genetically distinct (Cook et al, in prep; Mezhzherin and Serbenyuk, 1992; Wakana et al., 1996), multiple examples of interspecific hybridization demonstrate a lack of complete reproductive isolation. Lack of chromosomal differentiation among species of *Clethrionomys* (Gamperl, 1982; Nadler et al., 1976; Obara et al, 1995; Rausch and Rausch, 1975) may facilitate the retention of reproductive compatibility among congeneric arvicolines (Chaline, 1987). Though laboratory attempts to breed *C. gapperi* and *C. rutilus* have been unsuccessful (Matthey,

1953; Zimmerman, 1965), it has been suspected that a potential for hybridization exists (MacDonald and Cook, 1996; Nadler et al., 1976).

Origin of the hybrid zone

The phylogeographic histories of *C. rutilus* and *C. gapperi* suggest that genetic introgression in southeast Alaska is a result of post-glacial contact, and parallels the hybrid zone of *C. rutilus* and *C. glareolus* in Fennoscandia. Like the zone in Fennoscandia, introgression of the *rutilus* mitochondrial genome has occurred in a region believed to be recently deglaciated (13,500 YBP; Mann and Hamilton, 1995). North of the ice sheets during the Pleistocene, expanding *C. rutilus* populations from Beringia would have colonized recently deglaciated regions of southeast Alaska from the north, coming into contact with the probably already established *C. gapperi* populations (Mandryk, 1996; Runck and Cook, in prep). Low levels of mitochondrial divergence ($p=0.006$) between the hybrid *C. gapperi* and *C. rutilus* (Runck and Cook, in prep.) suggest that introgression is relatively recent, probably occurring since the late Pleistocene.

Phylogeographic studies have shown southeast Alaska to be a contact zone for other refugial mammalian lineages. Multiple divergent lineages in southeast Alaska of dusky shrews (*Sorex monticolus*; Cook et al., 2001) and black bears (*Ursus americanus*; Stone and Cook, 2000) suggest post-glacial colonization, and subsequent contact of these refugial lineages into the region. In addition, the long-tailed vole (*Microtus longicaudus*; Conroy and Cook, 2000), and ermine (*Mustela erminea*; Fleming and Cook, submitted)

appear to have recolonized this region from both Beringian and southern refugial populations, coming into contact in southeast Alaska.

Cytonuclear disequilibrium in the hybrid zone

Asymmetric introgression of the *rutilus* haplotype does not appear to be a result of stochastic processes, because all specimens of *C. gapperi* examined from the hybrid zone possessed the haplotype diagnostic of *C. rutilus*, and introgression of the *C. gapperi* genome into *C. rutilus* was not observed. Dispersal of females, especially in expanding populations, will mediate asymmetrical introgression, distributing the matrilineal mitochondrial genome, and in some instances result in fixation of the invading genome (Crespin et al., 1999; Gill, 1997; Hewitt, 1996; Lehman et al., 1991; Smith et al., 1989; Wilson and Bernatchez, 1998). The expanding population of *C. rutilus* from the north, perhaps using the Stikine River valley as a corridor, may have encountered the already established population of *C. gapperi*. Colonization routes into southeast Alaska were available at an earlier time from the south (Mandryk, 1996), and molecular data suggest *C. gapperi* have existed in the region longer than *C. rutilus* (Runck and Cook, in prep.).

Behavioral interactions may further affect the dispersal and reproductive success of these two species in their contact zone. In behavioral experiments, female *C. rutilus* demonstrated more defensive responses to conspecific males than towards *C. gapperi*, (Murie and Dickinson, 1973). Therefore, interactions of colonizing female *C. rutilus* towards *C. gapperi* may be dictating the direction of gene flow across the species' boundary. Biased backcrossing of female F1 hybrids with *C. gapperi* males may have

advanced the mtDNA genome into the *C. gapperi* populations, allowing the *rutilus* mtDNA to spread 80 km south of the nuclear and morphological boundary.

Physiographic and ecological barriers can further influence the location and dynamics of hybrid zones (Barton and Hewitt, 1985; Hewitt, 1993; but see Moulin et al., 1996; Wyttenback et al., 1999). The northern extent of the *gapperi/rutilus* hybrid zone in southeast Alaska was found at Mallard Slough. Directly north of Mallard Slough is Leconte Glacier and Leconte Bay. This glacier and bay may act as a barrier to contemporary vole dispersal because voles are poor dispersers over water and ice (van Apeldoorn et al, 1992; Dewsbury et al., 1982; Getz, 1967). Barriers to the influx of the hybridizing population result in reduced frequency of nuclear alleles. Once the donor population is removed, less than 0.5% of nuclear alleles belonging to the donor hybridizing population will exist after 10 generations (Gyllensten et al., 1985), yet the mitochondrial genome will be present as long the lineage survives because it does not undergo recombination.

If these physiographic features are barriers to contemporary gene flow, the *rutilus* mitochondrial DNA will remain detectable, but nuclear alleles will become diluted over succeeding generations. Though geographic and genetic distances have not been extensively assessed in this hybrid zone, cytochrome *b* sequences from introgressed *C. gapperi* and *C. rutilus* north of the zone (Runck and Cook, in prep.) were not identical and possess minimal divergence, which may suggest that hybridization is historical. However, additional specimens and markers need to be analyzed to determine the dynamics of this hybrid zone. Use of microsatellite markers would provide insight on

gene flow within and adjacent to the hybrid zone, and could determine the presence of a physiographic barrier to gene flow.

Selection in the hybrid zone

The width of a hybrid zone is usually directly proportional to the strength of selection on the hybrids (Barton and Hewitt, 1981). Though mtDNA is believed to flow across species boundaries more readily than nuclear DNA (due to less selection pressure on the mitochondrial genome), mtDNA does not encode all the necessary proteins involved in replication (Brown, 1983; O'Brien et al, 1980; Takahata and Slatkin, 1984). Therefore, some level of selection on this genome with respect to its interaction with the nuclear genome is expected (Lamb and Avise, 1986). Although the strength of selection cannot be measured in this hybrid zone, selection against hybrids can be indirectly assessed. For instance, age classes can indirectly estimate selection strength (Barton and Hewitt, 1985; Lamb and Avis, 1986). A deficiency of the older age groups was not observed in the hybrid zone, indicating little evidence of reduced viability with age.

Sterility or some level of inviability of the F1 heterogametic sex (males in mammals) is relatively common (Haldane, 1922; Zeng and Singh, 1993). Male sterility has been documented for several F1 mammalian hybrids (Baker et al., 1989), including hybrids of *C. gapperi* and *C. glareolus* (Grant, 1974) and hybrids of *C. rutilus* and *C. glareolus* (Spannhof, 1959; Zimmerman, 1965). In this contact zone there may be reduced fertility of males, but the presence of adult male hybrids indicated that hybrids are not inviable. Without strong selection acting against cytonuclear heterozygotes, complete mitochondrial introgression into another genome can occur within decades

(Takahata and Slatkin, 1984). Complete mitochondrial introgression has been documented in the golden-winged warbler (*Vermivora chrysoptera*; Gill, 1997), and has potentially occurred in this *Clethrionomys* hybrid zone as well.

Further analyses, such as geographic association with nuclear loci, could elucidate the dynamics of this hybrid zone and explain the pattern of asymmetric introgression. In addition, detailed analyses of this zone could provide insight into strength of selection, dispersal of parental and hybrid populations, time of origin, and the influence of physiographic features on dispersal. Additional analyses across the range of the contact zone in Canada may help determine whether post-glacial contact had the same effects on populations across their distribution.

CONCLUSIONS

C. rutilus and *C. gapperi* in southeast Alaska are another example of how late Pleistocene climate fluctuations have influenced contemporary species distributions and interactions. Separated by the Laurentide and Cordilleran ice sheets, the recent trans-Beringian colonizer *C. rutilus* (Gromov and Polyakov, 1977) colonized southeast Alaska from the north, and *C. gapperi* colonized from southern refugia as the ice sheets receded (Hibbard et al., 1965). Contemporary parapatric distributions of these two species meet near the Stikine River in southeast Alaska. Analyses of the nuclear MYH2 sequence and post-palatal bridge were concordant and indicated that LeConte Bay was the physiographic feature separating these two species. Asymmetric introgression of the mitochondrial genome of *C. rutilus* was observed in *C. gapperi* up to 80 km south of Leconte Bay. Because asymmetric introgression occurred in all *C. gapperi* in the hybrid

zone and the hybrid zone is wide, introgression does not appear to be recent and may date back to colonization of *C. rutilus* and *C. gapperi* into southeast Alaska. Asymmetric introgression suggests that interspecific interactions have not affected both species in the same way. Behavioral, ecological, or genetic factors are either advancing the *C. rutilus* or restricting the influx of *C. gapperi* mitochondrial genomes.

ACKNOWLEDGMENTS

We thank Brandy Jacobsen for her laboratory assistance and Tom LeCroy for his technical support in the UAF Core Sequencing Lab. John Bender II, Richard Runck, Leigh Dickey, Howie Chen, Allison Bidlack, Tom Seaton, Steve MacDonald, Doug Clark, and Laura Elze provided excellent help with fieldwork. Special thanks to Cole Crocker-Bedford, Mike Brown, Ed Grossman, Joe Spicanni, Peg Robertson, Lynn Atkins and Scott Posner for assistance with logistics while collecting in southeast Alaska. Karen Stone and Abel Bult offered valuable assistance with the analyses. This work was funded by the US Fish and Wildlife Service, USDA Forest Service, and the National Science Foundation.

LITERATURE CITED

Anderson, S. 1960. The baculum in microtine rodents. University of Kansas Publications Museum of Natural History, 12:181-216.

van Apeldoorn, R., W. R. Ostenbrink, A. van Winden, and F. F. Zee. 1992. Effects of habitat fragmentation on the bank vole *Clethrionomys glareolus* in an agricultural landscape. Oikos, 65:265-274.

Avise, J. C. 1994. Molecular Markers, Natural History, and Evolution. New York, Chapman and Hall.

Avise, J. C., and D. Walker. 1998. Pleistocene phylogeographic effects on avian populations and the speciation process. Proceedings of the Royal Society of London, 265:457-463.

Baker, R. J., S. K. Davis, R. D. Hamilton, and M. J. Van Den Bussche. 1989. Ribosomal-DNA, mitochondrial-DNA, chromosomal, and allozymic studies on a contact zone in the pocket gopher, *Geomys*. Evolution, 43:63-75.

Barton, N. H., and G. M. Hewitt. 1981. The genetic basis of hybrid inviability between two chromosomal races of the grasshopper *Podisma pedestris*. Heredity, 47:367-383.

Barton, N. H., and G. M. Hewitt. 1985. Analysis of hybrid zones. Annual Review of Ecology and Systematics, 16:113-148.

Barton, N. H., and G. M. Hewitt. 1989. Adaptation, speciation and hybrid zones. Nature, 341:497-503.

Bee, J. W., and E. R. Hall. 1956. Mammals of the North Slope. University of

Kansas Publications Museum of Natural History, 8:1-309.

Bell, D. A. 1996. Genetic differentiation, geographic variation and hybridization in gulls of the *Larus glaucescens-occidentalis* complex. Condor, 98(3):527-546.

Brown, W. M. 1983. Evolution of animal mitochondrial DNA. Pp. 62-88 in Evolution of Genes and Proteins (M. Nei, and R. K. Koehn, eds.). Sinauer, Sunderland, Massachusetts.

Canham, R. P., and D. G. Cameron. 1972. Variation in the serum proteins of the red-backed mice *Clethrionomys rutilus* and *C. gapperi* and its taxonomic significance. Canadian Journal of Zoology, 50:217-227.

Chaline, J. 1987. Arvicolid data (Arvicolidae, Rodentia) and evolutionary concepts. Evolutionary Biology, 21:237-310.

Conroy, C. J., and J. A. Cook. 2000. Phylogeography of a post-glacial colonizer: *Microtus longicaudus* (Rodentia: Muridae). Molecular Ecology 9:165-175.

Conroy, C. J., J. R. Demboski, and J. A. Cook. 1999. Mammalian biogeography of the Alexander Archipelago of Southeast Alaska: a north temperate nested fauna. Journal of Biogeography, 26:343-352.

Cook, J. A., A. L. Bidlack, C. J. Conroy, J. R. Demboski, M. A. Fleming, A. M. Runck, K. D. Stone, and S. O. MacDonald. 2001. A phylogeographic perspective on endemism in the Alexander Archipelago. Biological Conservation, 97:215-227.

Cook, J. A., A. M. Runck, and C. J. Conroy. in preparation. Historical biogeography at the crossroads of the northern continents: molecular

phylogenetics of red-backed voles (*Rodentia*: *Arvicolidae*).

Corbet, G. B. 1978. The Mammals of the Palaearctic Region: a taxonomic review. Cornell University Press, New York.

Crespin, L., P. Berrebi, and J. Lebreton. 1999. Asymmetrical introgression in a freshwater fish hybrid zone as revealed by a morphological index of hybridization. Biological Journal of the Linnean Society, 67(1):57-72.

Dansgaard, W., S. J. Johnsen, H. B. Clausen, D. Dahl-Jensen, N. S. Gundestrup, G. U. Hammer, C. S. Hvidberg, J. P. Steffensen, E. E. Sveinbjornsdottir, J. Jouzel, and G. Bond. 1993. Evidence for general instability of past climate from a 250-kyr ice-core. Nature, 364:218-220.

Dewsbury, D., D. J. Baumgardner, D. K. Sawrey, and D. G. Webster. 1982. The adaptive profile: comparative psychology of red-backed voles. Journal of Comparative and Physiological Psychology, 96:649-660.

Fleming, M. A., and J. A. Cook. submitted. Phylogeography of endemic ermine (*Mustela erminea*) in southeast Alaska. Journal of Mammalogy.

Gamperl, R. 1982. Chromosomal evolution in the genus *Clethrionomys*. Genetica, 57:193-197.

Getz, L. L. 1967. Response of selected small mammals to water. Occasional Papers of the University of Connecticut Biological Science Series, 1:71-81.

Gill, F. B. 1997. Local cytonuclear extinction of the golden-winged warbler. Evolution, 51:519-525.

Graham, R. W. 1976. Late Wisconsin mammalian faunas and environmental

gradient of the eastern United States. Paleobiology, 2:343-350.

Grant, P. R. 1974. Reproductive compatibility of voles from separate continents (Mammalia: *Clethrionomys*). Journal of Zoology of London, 174:245-254.

Greenland Ice-core Project Members 1993. Climate instability during the last interglacial period recorded in the GRIP ice core. Nature, 364:203-207.

Gromov, I. M., and I. Y. Polyakov. 1977. Voles (Microtinae), Fauna of the USSR, Mammals, Volume II, No. 8. Smithsonian Institution Libraries and the National Science Foundation, Washington, D. C.

Gyllensten, U., D. Wharton, and A. C. Wilson. 1985. Maternal inheritance of mitochondrial DNA during backcrossing of two species of mice. Journal of Heredity, 76:321-324.

Haldane, J. B. S. 1922. Sex ratio and unisexual sterility of hybrid animals. Journal of Genetics, 12:101-109.

Hall, E. R. 1981. Mammals of North America. John Wiley and Sons, New York.

Hamilton, W. J. 1946. A study of the baculum in some North American Microtinae. Journal of Mammalogy, 27:378-387.

Heaton, T. H., S. L.Talbot, and G. F. Shields. 1996. An Ice Age refugium of large mammals in the Alexander Archipelago, Southeastern Alaska. Quaternary Research, 46:186-192.

Hewitt, G. M. 1988. Hybrid zones – natural laboratories for evolutionary studies. Trends in Ecology and Evolution, 3:158-167.

Hewitt, G. M. 1993. After the ice: *Parallelus* meets *Erythropus* in the Pyrenees.

Pp. 140-146 in Hybrid Zones and the Evolutionary Process (R. G. Harrison, ed.). Oxford University Press, New York, USA.

Hewitt, G. M. 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. Biological Journal of the Linnean Society, 58:247-276.

Hibbard, C. W., C. E. Ray, D. E. Savage, D. W. Taylor, and J. E. Guilday. 1965. Quaternary mammals of North America. Pp. 509-525 in The Quaternary of the United States (H. E. Wright, Jr., and D. G. Frey, eds.). Princeton University Press, Princeton, New Jersey.

Hooper, E. T., and B. S. Hart. 1962. A Synopsis of Recent North American Microtine Rodents. Miscellaneous Publications Museum of Zoology University of Michigan, 20:181-216.

Klein, D. R., 1965. Postglacial distribution patterns of mammals in the southern coastal regions of Alaska. Journal of the Arctic Institute of North America, 18:7-20.

Lamb, T., and J. C. Avise. 1986. Directional introgression of mitochondrial DNA in a hybrid population of tree frogs: the influence of mating behavior. Proceedings of the National Academy of Sciences USA, 83:2526-2530.

Lehman, N. E., K Hansen, L. D. Mech, R. O. Peterson, P. J. P. Gogan, and R. K. Wayne, 1991. Introgression of coyote mitochondrial DNA into sympatric North American gray wolf populations. Evolution, 45:104-119.

Lessa, E. P., and J. A. Cook. 1998. The molecular phylogenetics of tuco-tucos (genus *Ctenomys*, Rodentia: Octodontidae) suggests an early burst of

speciation. Molecular Phylogenetics and Evolution, 9:88-99.

Lowe, V. P. W. 1971. Root development of molar teeth in the bank vole (*Clethrionomys glareolus*). Journal of Animal Ecology, 40:49-61.

Lyons, L. A., T. F. Laughlin, N. G. Copeland, N. A. Jenkins, J. E. Womack, and S. J. O'Brien. 1997. Comparative anchor tagged sequences (CATS) for integrative mapping of mammalian genomes. Nature Genetics, 15:47-56.

MacDonald, S. O. and J. A. Cook. 1996. The land mammal fauna of southeast Alaska. Canadian Field-Naturalist, 110:571-598.

MacPherson, A. H. 1965. The origin of diversity in mammals of the Canadian arctic tundra. Systematic Zoology, 14:153-173.

Mandryk, C. A. S. 1996. Late Wisconsin deglaciation of Alberta: processes and paleogeography. Quaternary International, 32:79-85.

Mann, D. H., and T. D. Hamilton. 1995. Late Pleistocene and Holocene paleoenvironments of the north pacific coast. Quaternary Science Review 14:449-471.

Martell, A. M. and W. A. Fuller. 1978. Comparative demography of *Clethrionomys rutilus* in taiga and tundra in the low Arctic. Canadian Journal of Zoology, 57:2106-2120.

Matthey, R. 1953. Les chromosomes des Muridae. Révision critique et matériaux nouveaux pour servir á l'histoire de l'évolution chromosomique chez ces rongeurs. Rev. Suisse. Zool., 60:225-283.

Mezhzherin, S. V., and M. A. Serbenyuk. 1992. Biochemical variability and

genetic divergence of Palaeartic Arvicolidae. Genus of red-backed voles, *Clethrionomys* Tilesius, 1850. Russian Journal of Genetics, 28:217-226.

Mihok, S. 1980. Ageing young *Clethrionomys gapperi* with M2 tooth characteristics. Canadian Journal of Zoology, 58:2280-2281.

Miller, S. A., D. D. Dykes, and H. F. Polesky. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acid Research, 16:1215.

Moulin, N. L., A. Wyttenback, H. Brünner, J. Goudet, and J. Hausser. 1996. Study of gene flow through a hybrid zone in the common shrew (*Sorex araneus*) using microsatellites. Hereditas, 125:159-168.

Murie, J. O., and D., Dickinson 1973. Behavioral interactions between two species of red-backed vole (*Clethrionomys*) in captivity. Canadian Field Naturalist, 87:123-129.

Nadler, C. F., V. R. Rausch, E. A. Lyapunova, R. S. Hoffmann, and N. N. Voronstov. 1976. Chromosomal banding patterns of the Holarctic rodents, *Clethrionomys rutilus* and *Microtus oeconomus*. Zhurnal Säugetierkunde, 41:137-146.

O'Brien, T. W., N.D. Denslow, T. O. Harville, R. A. Hessler, and D. E. Matthews. 1980. Functional and structural roles of proteins in mammalian mitochondrial ribosomes. Pp. 301-305 in The Organization and Expression of the Mitochondrial Genome (A. Kroon, and C. Saccone, eds.). Elsevier, North Holland, New York.

Obara, Y., H. Kusakabe, K. Miyakoshi, and S. Kawada. 1995. Revised karyotype of the Japanese northern red-backed vole, *Clethrionomys rutilus mikado*. Journal of the Mammalogical Society of Japan, 20:125-133.

Randi, E. and A. Bernard-Laurent. 1999. Population genetics of a hybrid zone between the red-legged partridge and rock partridge. The Auk, 116:324-337.

Rausch, R. L. and V. R. Rausch. 1975. Relationships of the red-backed vole, *Clethrionomys rutilus* (Pallas), in North America: karyotypes of the subspecies *dawsoni* and *albiventer*. Systematic Zoology, 24:163-170.

Runck, A. M., and J. A. Cook. in preparation. A phylogeographic perspective on the endemic red-backed voles of Southeast Alaska. Canadian Journal of Zoology.

SAS Institute Inc. 1996. The SAS system for window, Release 6.12. SAS Institute Inc., Cary, North Carolina.

Sattler, G. D., and M. J. Braun. 2000. Morphometric variation as an indicator of genetic interactions between black-capped and Carolina chickadees at a contact zone in the Appalachian Mountains. The Auk, 117(2):427-444.

Smith, D. R., O. R. Taylor, and W. M. Brown, 1989. Neotropical Africanized honeybees have African mitochondrial DNA. Nature, 339:213-215.

Smith, M. F., and J. L. Patton. 1993. The diversification of South American murid rodents: evidence from the mitochondrial DNA sequence data for the akodontine tribe. Biological Journal of the Linnean Society, 50:149-177.

Spannhof, L. 1959. Histochemische untersuchungen zur sterilität bei männlichen säugerbastarden (Artkrezung der Rötelmäuse *Clethrionomys glareolus* x *Cl. rutilus*).

Verh Stsch Zool Des Zoll Anz Suppl 23:99-107.

Stone, K. D. and J. A. Cook. 2000. Phylogeography of black bears (*Ursus americanus*) of the Pacific Northwest. Canadian Journal of Zoology, 78:1218-1223.

Swofford, D. L. 2000. PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods). Sinauer Associates, Sunderland, Massachusetts.

Takahata, N., and M. Slatkin. 1984. Mitochondrial gene flow. Proceedings of the National Academy of Sciences, 81:1764-1767.

Tegelström, H. 1987. Transfer of mitochondrial DNA from the northern red-backed vole (*Clethrionomys rutilus*) to the bank vole (*C. glareolus*). Journal of Molecular Evolution, 24:218-227.

Tremblay, O. N., and J. D. Schoen. 1999. Molecular phylogeography of *Dryas integrifolia*: glacial refugia and postglacial recolonization. Molecular Ecology, 8: 1187-1198.

Tupikova, N. V., G. A. Disorova, and E. A. Konovalova. 1968. A method of age determination in *Clethrionomys*. Acta Theriologica, 20:99-115.

Vanlerberghe, F. B., P. Dod, M. Bellis, and F. Bonhomme. 1986. Absence of Y chromosome introgression across the hybrid zone between *Mus musculus* and *Mus domesticus*. Genetic Research, 48:191-197.

Wakana, S., M. Sakaizumi, K. Tsuchiya, M. Asakawa, S. H. Han, and H. Suzuki. 1996. Phylogenetic implications of variations in rDNA and mtDNA in red backed voles collected in Hokkaido, Japan, and in Korea. Mammal Study, 21:15-25.

Webb, T. III., and P. J. Bartlein. 1992. Global changes during the last 3 million

years: climatic controls and biotic responses. Annual Review of Ecology and Systematics, 23:141-173.

Wilson, C. C., and L. Bernatchez. 1998. The ghosts of hybrids past: fixation of arctic charr (*Salvelinus alpinus*) mitochondrial DNA in an introgressed population of lake trout (*S. namaycush*). Molecular Ecology, 7:127-132.

Wyttenback, A., Y. Narain, and K. Fredga. 1999. Genetic structuring and gene flow in a hybrid zone between two chromosome races of the common shrew (*Sorex araneus*, Insectivora) revealed by microsatellites. Heredity, 82:79-88.

Youngman, P. M. 1975. Mammals of the Yukon Territory. National Museum of Canada, Publication in Zoology, 10:1-192.

Zeng, L., and R. S. Singh. 1993. The genetic basis of Haldane's Rule and the nature of asymmetric hybrid male sterility among *Drosophila simulans*, *Drosophila mauritiana* and *Drosophila sechillia*. Genetics, 134:251-260.

Zimmerman, K. 1965. Art-hybriden bei Rötelmäusen. Z Säugetierkunde, 30:315-320.

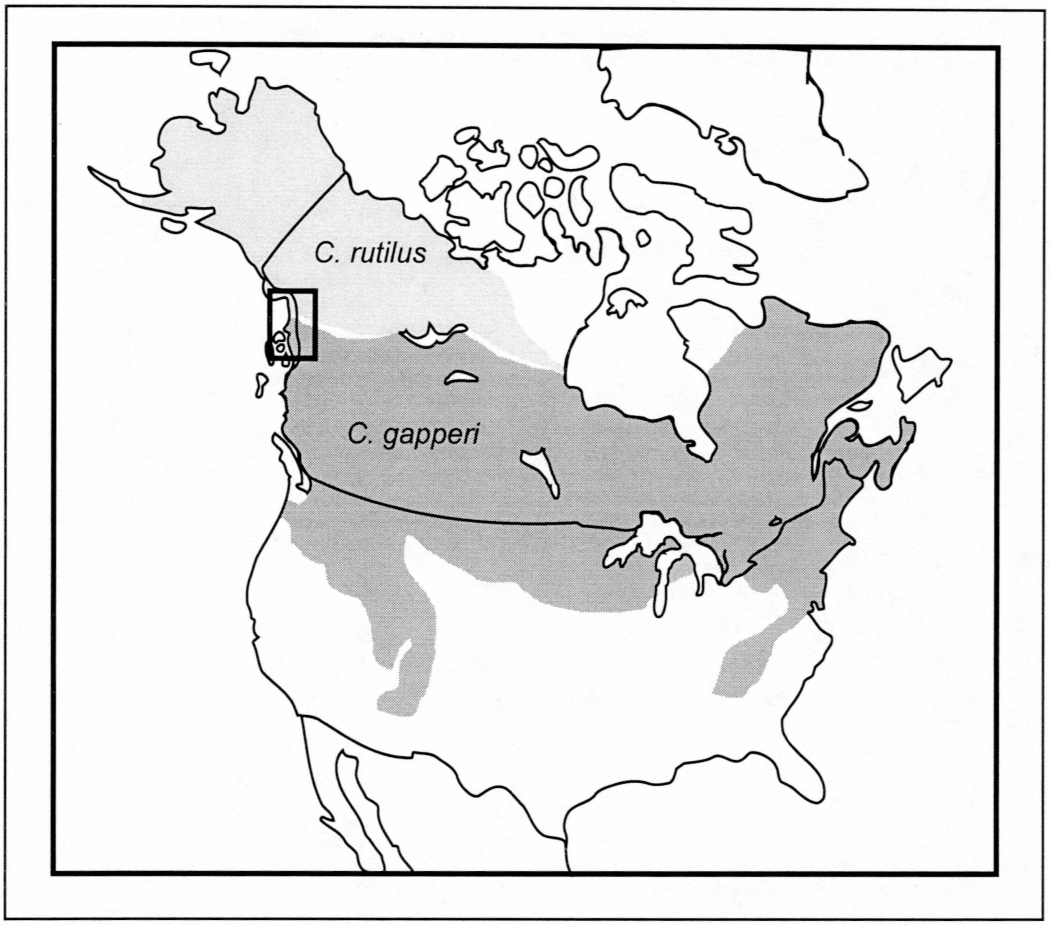


Figure 1. Distribution of *Clethrionomys rutilus* and *C. gapperi* in North America, modified from Corbet (1974) and Hall (1981). High-lighted is southeast Alaska where this study focused.

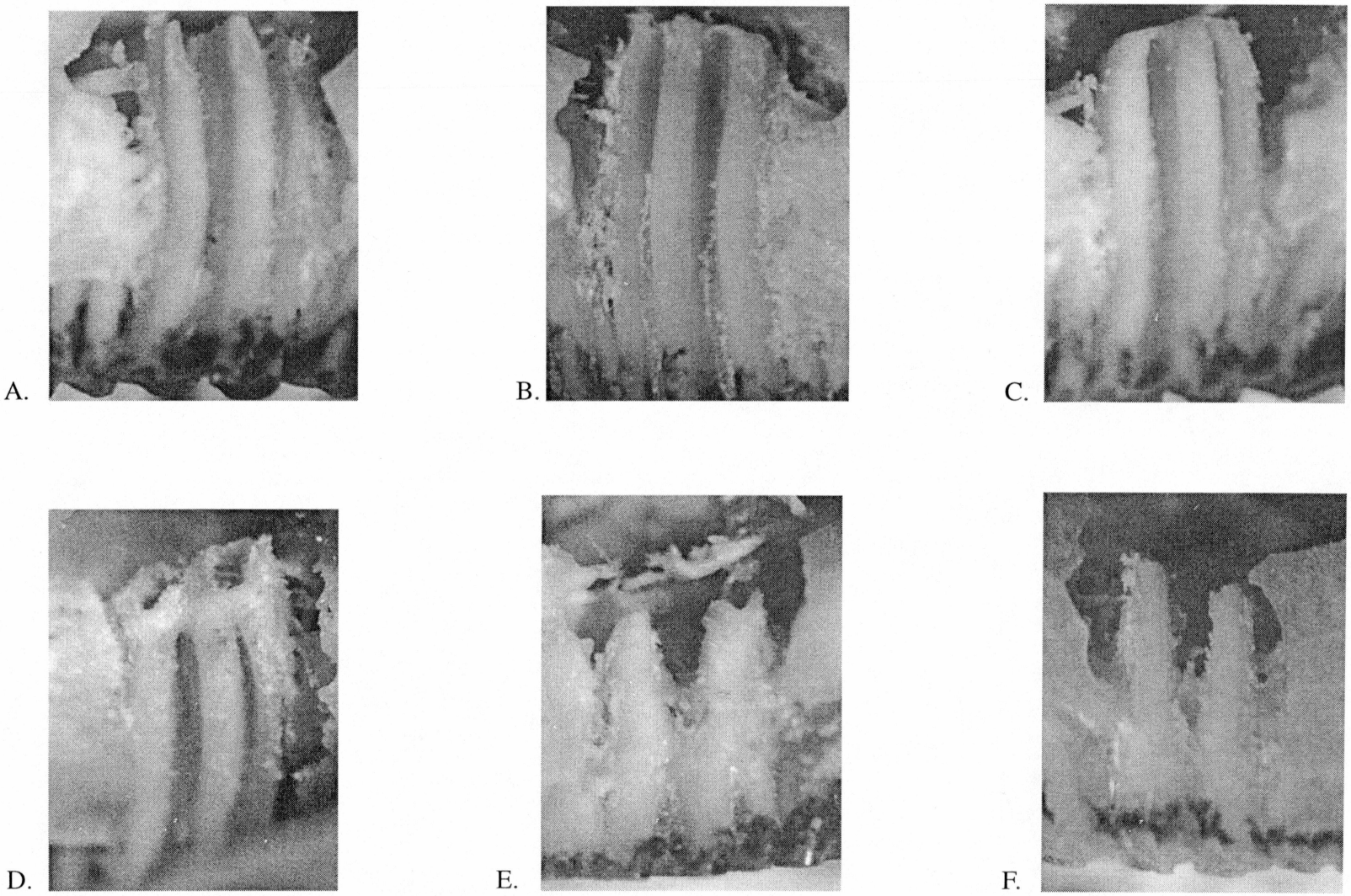
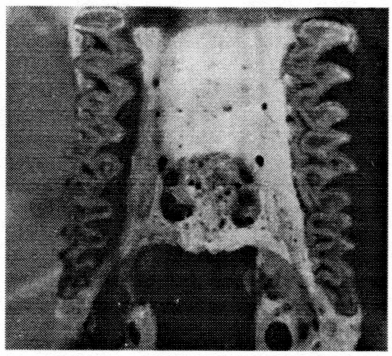


Figure 2. Six age catagories as determined by second upper molar (M2) root development in *Clethrionomys*. Labial aspect shown and anterior is to the right. A. Anterior groove open. B. Anterior groove closing. C. Anterior groove closed. D. Neck forming. E. Roots formed, < 1mm. F. Roots formed, > 1mm.

A.



B.

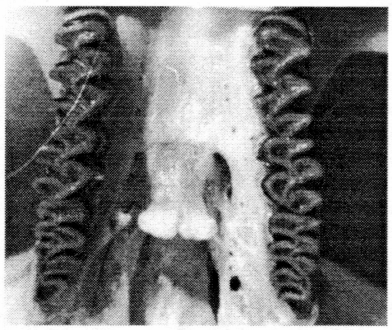


Figure 3. Ossification of the post-palatal bridge. Ventral veiw of cranium; posterior is to the bottom. A. Complete, with medial shelf connected to lateral parts of the palate. Diagnostic of *C. gapperi*. B. Incomplete, with medial shelf not connected to lateral parts of the palate. Diagnostic of *C. rutilus*.

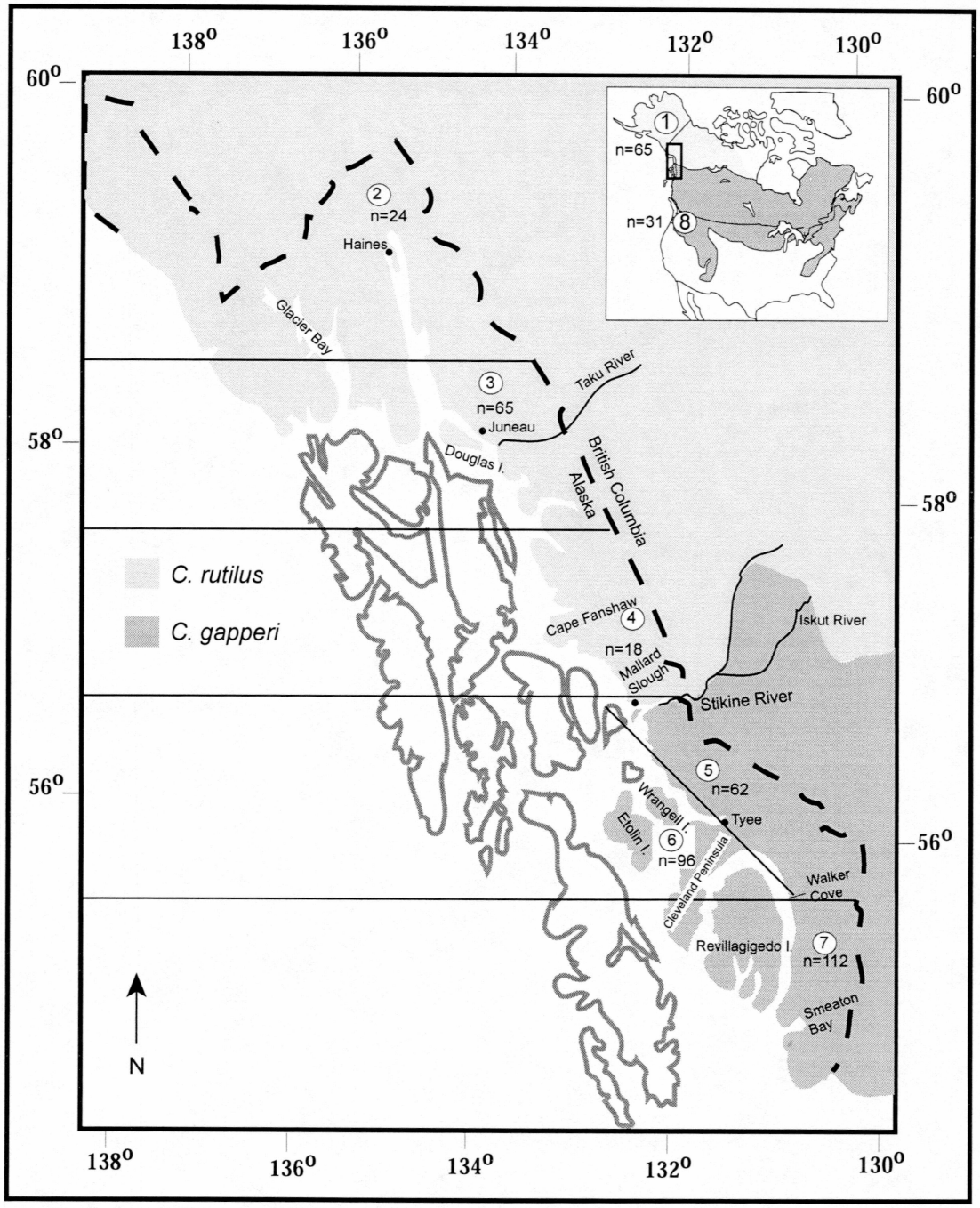


Figure 4. Eight geographic groups created (by latitude) to analyze relationship of age and sex on the post palatal bridge. Sample sizes are indicated.

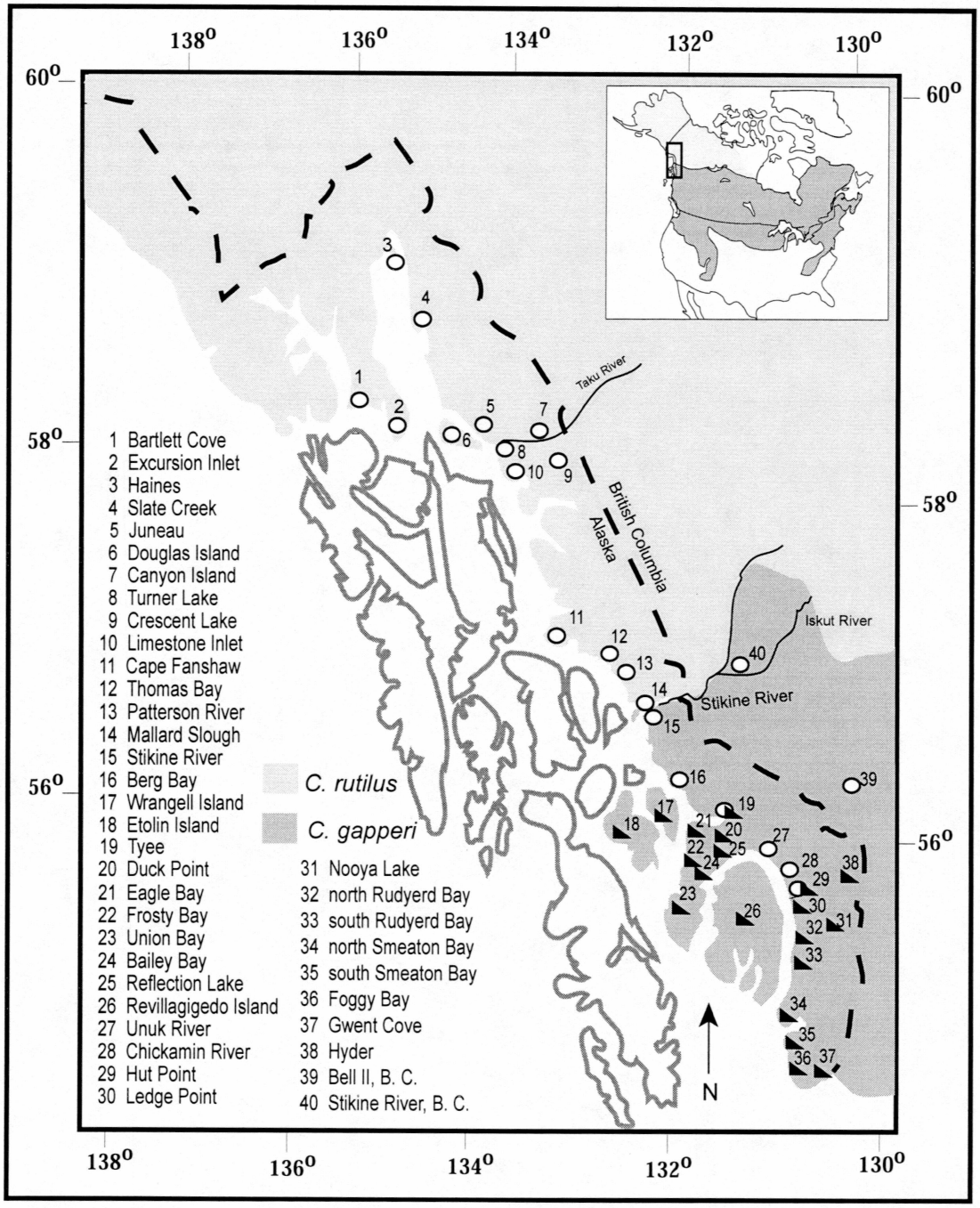


Figure 5. Sample sites and distributions of the cytochrome *b* haplotypes of C. rutilus (○) and C. gapperi (◣)in southeast Alaska and Bristish Columbia.

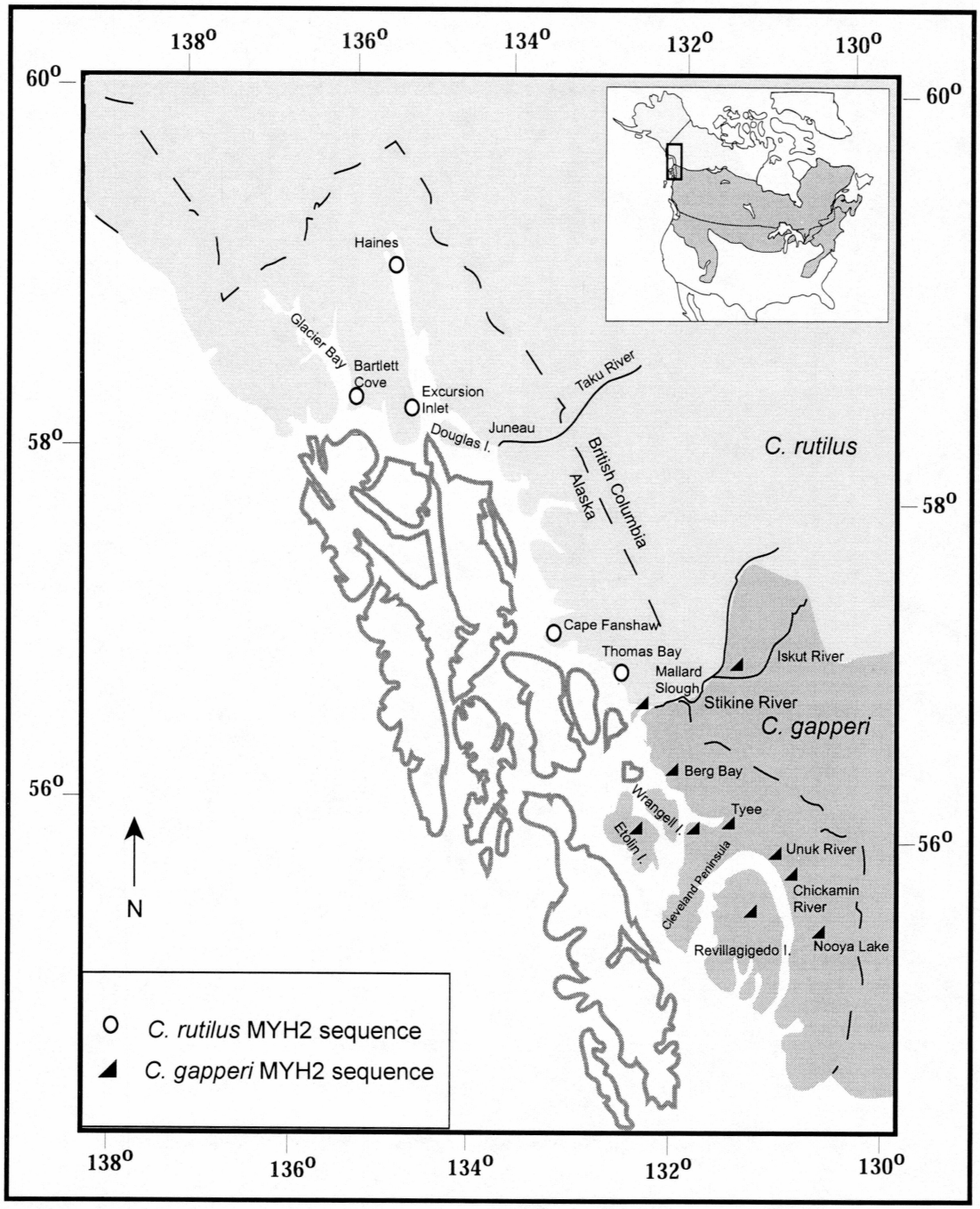


Figure 6. Sample localities and distributions of the nuclear MYH2 sequences in southeast Alaska.

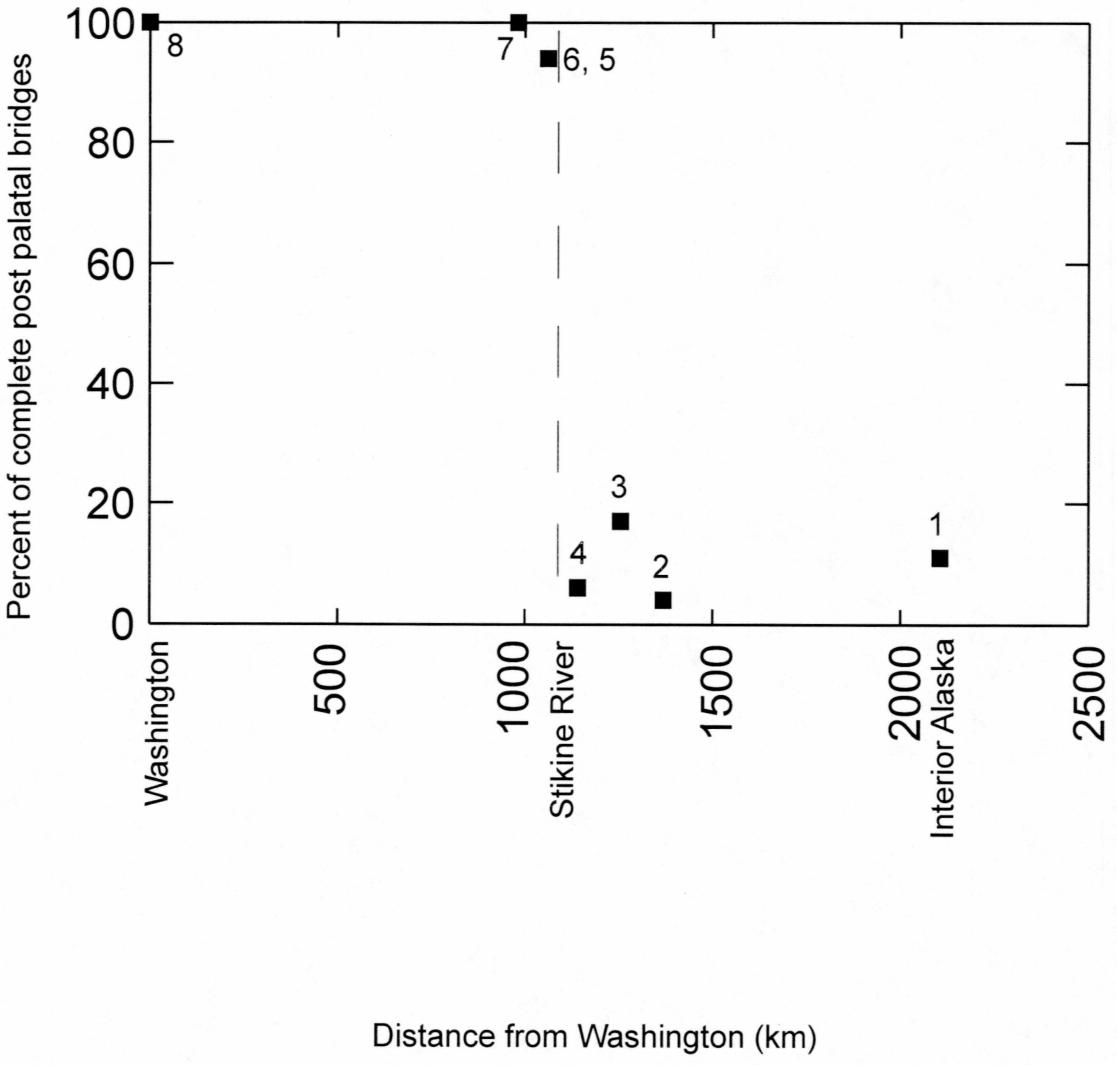


Figure 7. Frequencies of complete post palatal bridges by geographic location. Numbers correspond with locations in Figure 4.

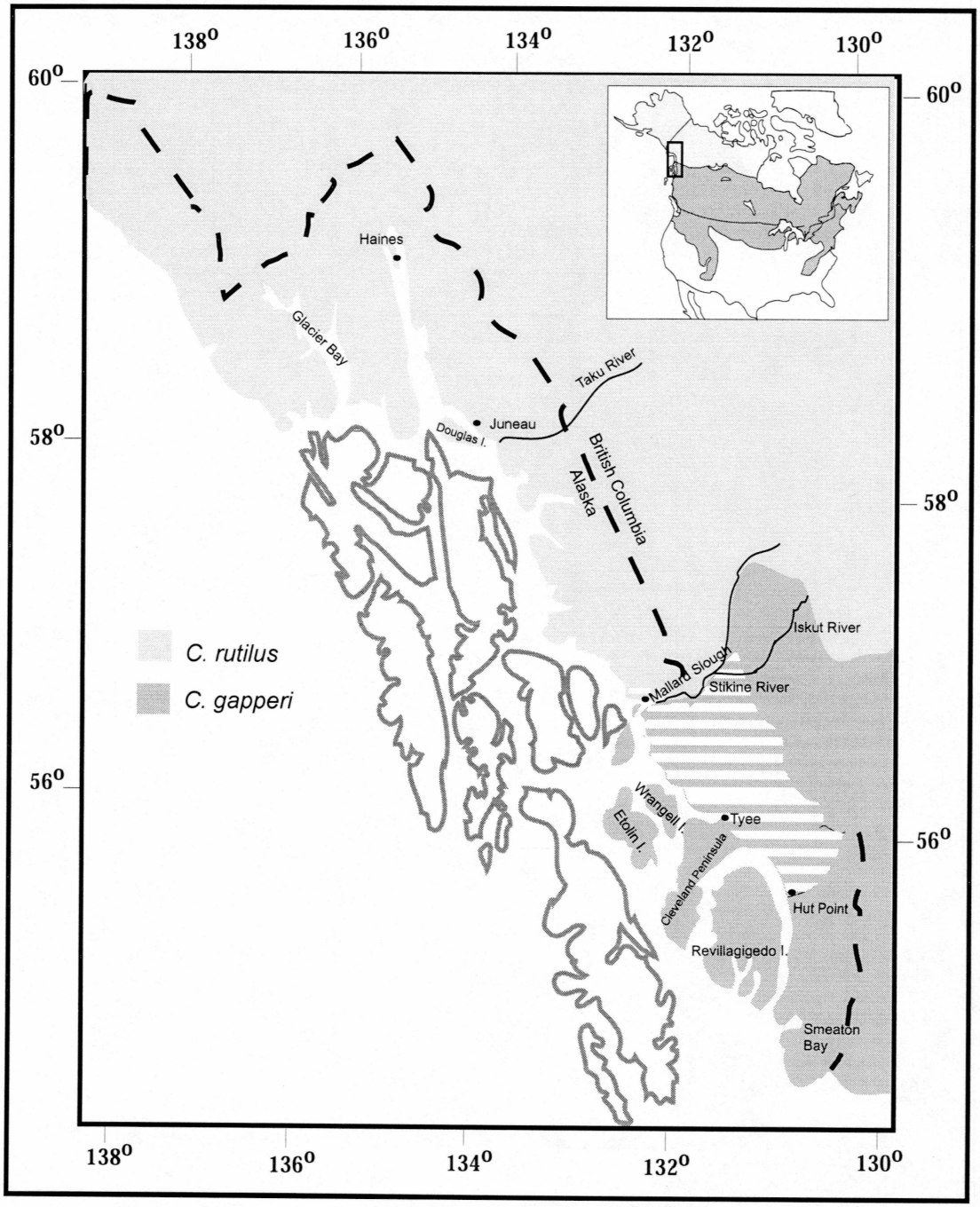


Figure 8. Map of southeast Alaska showing the lack of concordance of the three markers. Striped area indicates that specimens had the post palatal bridge morph and nuclear MYH2 sequence diagnostic for *C. gapperi* and the cytochrome *b* haplotype diagnostic for *C. rutilus*.

Appendix I. Specimens and their localities used in RFLP analysis of cytochrome *b*, and sequence variation of the MYH2 nuclear intron.

Locality and species	**AF**	**Cytochrome *b* haplotype**	**AF**	**MYH2**
Clethrionomys rutilus				
Finland	3130[1]	*rutilus*	3130	*rutilus*
Russia	3786[1]	*rutilus*	3786	*rutilus*
Denali Nat. Park	4853[1]	*rutilus*	4853	*rutilus*
Bartlett Cove	6486[1], 6508[1]	*rutilus*		
Excursion Inlet	17262, 17263[1], 17264, 17607, 17612	*rutilus*	2379, 17612	*rutilus*
Haines	21530, 21531[1], 21535, 22099, 28814-28819	*rutilus*	21530	*rutilus*
Slate Creek	21743	*rutilus*		
Juneau	1816[1], 7052[1]	*rutilus*		
Douglas Island		*rutilus*		
Canyon Island	8270, 8279[1]	*rutilus*		
Turner Lake	8484	*rutilus*		
Crescent Lake	8315[1]	*rutilus*		
Limestone Inlet	19686	*rutilus*		
Cape Fanshaw	19606	*rutilus*		
Thomas Bay	5278[1]	*rutilus*	5278	*rutilus*
Patterson River	21919-21922, 21925-21927, 21928[1], 21933, 21934, 21935, 21936[1], 21937, 25630	*rutilus*	19606	*rutilus*
Clethrionomys gapperi				
Mallard Slough	21400, 21401, 21402, 21403, 21409, 21413-21419, 21420[1], 21421-21425, 21450-21457, 21483-21486	*rutilus*	21400, 21457, 21483	*gapperi*

Appendix I. Specimens and their localities used in RFLP analysis of cytochrome *b*, and sequence variation of the MYH2 nuclear intron (continued).

Locality and species	**AF**	**Cytochrome *b* haplotype**	**AF**	**MYH2**
Stikine River area	2623[1], 2644[1], 2655[1], 2812[1], 2823[1], 2825[1]	*rutilus*		
Berg Bay	21801, 21802, 21811, 21818[1], 21819	*rutilus*	21818	*gapperi*
Wrangell Island	2589[1], 2780[1], 25631, 25632, 25633, 25658-25660, 25766-25771[1], 25772[1], 25673, 25676-25678, 25768, 25778-25789, 25791-25806, 25813-25819	*gapperi*	21483	*gapperi*
Etolin Island	2567, 2576, 2577, 2601-2604, 2619, 14798, 15665, 15782, 15789, 15791[1], 15806[1],15808-15810, 15815, 15817, 15821-15823, 15825, 15826, 25831[1]-25834, 25840, 25841, 25844, 25845, 25849-25851, 25855, 25856, 25865[1], 25868-25872[1], 25873, 25878, 25879, 25885, 25889-25893, 25895[1]	*gapperi*	15789	*gapperi*
Tyee	25921, 25925-25931[1], 25939, 25955, 25956, 25961[1]-25967, 25981, 25982, 25998-25600[1], 25601-26003, 26029, 26030, 26032,	*rutilus* (4) *gapperi* (25)	25938	*gapperi*
Salmon River	21943[1] , 21944[1]	*gapperi*		
Duck Point	26033[1], 26035[1], 26036, 26038-26043, 26050-26056, 26067-26076, 26080-26086, 26090-26094, 26105, 26106	*gapperi*		
Eagle Bay	26050, 26103[1]	*gapperi*		
Hoya Creek	19507-19509[1], 19510-19515, 24336	*gapperi*		
Frosty Bay	21835-21840, 21844-21854, 21865-21872, 22902[1]-22906	*gapperi*	21872, 21837	*gapperi*

Appendix I. Specimens and their localities used in RFLP analysis of cytochrome *b*, and sequence variation of the MYH2 nuclear intron (continued).

Locality and species	**AF**	**Cytochrome *b* haplotype**	**AF**	**MYH2**
Union Bay	4719-4727, 4729, 4739, 4749, 4750, 4751, 4760, 4769, 4770	*gapperi*		
Bailey Bay	1873[1]	*gapperi*		
Reflection Lake	29130, 29141, 29154[1], 29155, 29184	*gapperi*		
Revillagigedo Island	4326, 4786[1], 4788[1], 4995[1], 8320, 8321, 12293[1], 12294[1], 29545[1], 29577, 29578, 29586, 29588, 29589[1], 29599, 29604-29607, 29609	*gapperi*	4786	*gapperi*
Unuk River	4358, 4359, 4363-4365, 4370, 4377, 4405-4407, 4421, 4423, 4425[1], 4427, 4436, 4437, 4445, 4447, 4462, 4463, 4465[1], 4471, 4472, 4478, 4479, 4892, 4894-4896, 4898	*rutilus*	4425	*gapperi*
Chickamin River	4904, 4923, 4924[1], 4926, 4927[1], 4928, 4954, 4955, 4969	*rutilus*	4926	*gapperi*
Hut Point	29419, 29420, 29421[1], 29422[1]-29427, 29433-29436, 29462, 29463[1], 29464[1]	*rutilus* (4) *gapperi* (10)		
Ledge Point	29411-29416[1], 29456[1]	*gapperi*		
Nooya Lake	[1]22585, 22856	*rutilus*	22585	*gapperi*
north Rudyerd Bay	29299-29302[1], 29303[1], 29304-29307, 29311, 29312[1]-29314, 29352-29356, 29374, 29375[1]-29378, 29401	*gapperi*		
south Rudyerd Bay	29347-29349, 29369, 29370, 29396, 29397,	*gapperi*		
north Smeaton Bay	29271[1]-29273, 29290, 29291	*gapperi*		
south Smeaton Bay	29280, 29284[1], 29293	*gapperi*		

Appendix I. Specimens and their localities used in RFLP analysis of cytochrome *b*, and sequence variation of the MYH2 nuclear intron (continued).

Locality and species	**AF**	**Cytochrome *b* haplotype**	**AF**	**MYH2**
Foggy Bay	4270[1]	*gapperi*		
Gwent Cove	26193, 26552[1], 26556, 26571, 26572, 26573, 26574, 26578[1], 26597	*gapperi*		
Hyder	30186, 30187[1]	*gapperi*		
Stikine River, B. C.	12748[1], 12749, 12770-12772, 12778[1], 12779[1], 21992[1], 21993-22000, 22919, 22920[1], 22921	*rutilus*	12748	*gapperi*
Bell II, B. C.	29646, 29647, 29649, 29650, 29666-29669, 29672, 29673, 29681	*rutilus*		
Minnesota	17697[1]	*gapperi*	17697	*gapperi*

[1]Cytochrome *b* sequence data.

Appendix II. *Clethrionomys rutilus* and *C. gapperi* specimens that were aged using M^2 root development and scored for completeness of the post-palatal bridge. All specimens are deposited at the University of Alaska Museum (UAM, or AF). Numbers correspond to localities in Figure 4.

1. Interior Alaska (n=65)

Rock Creek, Denali: UAM 24610, 24614, 24616-24620, 24623, 24624, 24627, 24630, 24633, 24637, 24640, 24644, 24647, 24650, 24651, 24653, 24654, 24657, 24659, 24661-24664, 34137, 34164, 34246, 34250, 51526, 51526, 34247, 50594, 50546, 51530, 51460, 51468, 51470, 51474, 51478, 51484, 51488, 51495, 51496, 51503,51504, 51509, 51510, 51513, 51515, 51527, 51521, 50593; Fairbanks: UAM 22084, 22091, 22202, 22224, 22225, 22076, 50445, 51595, 51623, 51630, 51638, 32775, 2416.

2. Skagway area (n=24)

Chilkat Peninsula: UAM 50974, 50977, 50978,51022; Klukwan: UAM 31120-31122, 31125; Laughton Glacier: UAM 11976, 11977, 11989-11991; Taiya River area: UAM 11988, 14499-14504, 14508, 14509, 47558, 47559.

3. Juneau area (n=65)

Echo Cove: UAM 48856, 48858, 48859, 50956, 50957, 50959, 50960-50963, 50965, 50967, 50979-50981, 50983-50987, 50990-51004, 51006-51011, 51013; Turner Lake area: UAM 50652-50654, 50658, 36779, 36780, 36782-36790; Limestone Inlet: UAM 44610, 44612; Lynn Canal: UAM 14612, 34253, 34254; Powers Creek: UAM 50561, 50880, 50882.

Appendix II. *Clethrionomys rutilus* and *C. gapperi* specimens that were aged using M^2 root development, and scored for completeness of the post-palatal bridge. All specimens are deposited at the University of Alaska Museum (UAM or AF). Numbers correspond to localities in Figure 4 (continued).

4. Patterson River area (n=18)

Patterson River: UAM 48863, 48865-48868; Cape Fanshaw: UAM 44580-44584, 44586-44588, 44590, 44591, 44593, 44598, 44579.

5. Stikine River area (n=62)

Berg Bay: UAM 50293, 50294, 50296; Chickamin River: UAM 10360, 10361, 10363, 23923, 23924, 23926-23930; Farm Island: UAM 29984, 29986, 29987, 29989; Mallard Slough: UAM 50297, 50298, 50300, 50302-50305, 51024, 51018, 51020, 44592, 44579, 44591, 44610, 44612, 450487, 50490, 50472, 50480, 50491, 50489, 50483, 50479, 50481, 50488; Tyee: AF 25939, 25955, 26038, 26050; Unuk River: UAM 23918, 23573, 23591, 23920, 23532, 23547, 23553, 23562, 23563, 23603, 23551, 23474, 23488, 23493, 23505, 23919.

6. Cleveland Peninsula area (n=96)

Bailey Bay: 20591, 20593; Etolin Island: UAM 20640, 20649, 41649, 41646, 41648, 41885, 41886, 41887, 41890, 41889, 41891, 43129, 51963, 43132, 43133, AF 25844, 25849, 25855,25856, 25870, 25879, 25890, 25892, 25833, 25841, 25845; Frosty Bay: 50308, 50955, 50970, 50972, 50973, 50971, 50473, 50486, 50476, 50484, 50474, 50471, AF 21850; Hoya River: UAM 49987-49989, 52170; Wrangell Island: UAM 14821, 14823, 20766, 20767, 22980, 20673, 14820, 23058, 30719, 30883, 50109-50113, 50115-

Appendix II. *Clethrionomys rutilus* and *C. gapperi* specimens that were aged using M^2 root development, and scored for completeness of the post-palatal bridge. All specimens are deposited at the University of Alaska Museum (UAM or AF). Numbers correspond to localities in Figure 4 (continued).

50117, 25630, 25632, 25772, 25774, 25783, 25800, 25806, 25818, 25771, 25780, 25793, 25794, 25797, 25798,25802, 25803, 25813; Grant Creek: UAM 20598-2600; Reflection Lake: AF 29056, 29057,29059-29061, 29075, 29076, 29105, 29118, 29119, 29141, 29154, 29184.

7. Misty Fjords area (n=112)

Bond Bay: UAM 51145, 51146, 41140, 51132, 51133, 51152, 51143, 51141, 51148, 51131, 51158, 51159, 51160, 51162, 51142, 51150, 51135, 51134, 51136,51155, 51156, 51157; Foggy Bay: UAM 23414, 23428, 23429; Gwent Cove: AF 26191, 26194, 26195, 26556, 26571, 26572, 26574, 26575, 26579, 26588, 26596, 26597, 29397, 29412, 29414, 29415, 29419-29423, 29425, 29427, 29435, 29436; Walker Cove: Revillagigedo Island: UAM 14822, 23668, 23823, 23824, 23963, 30710-30714, 31844, 31845, 31877, 50877, AF 29225-29227, 29256, 29268, 29536, 29537, 29598; Smeaton Bay: AF 29272, 29272, 29290, 29291, 29280, 29284, 29339; Union Bay: UAM 23756, 23757, 23762, 23772, 23789, 23790, 23791, 23799, 23800, 46989; Rudyerd Bay: AF 29313, 29299-29302, 29304, 29306, 29346-29348, 29396; Port Stewart: UAM 51129, 41144, 51161, 51163, 51164, 51166, 51168.

Appendix II. *Clethrionomys rutilus* and *C. gapperi* specimens that were aged using M^2 root development, and scored for completeness of the post-palatal bridge. All specimens are deposited at the University of Alaska Museum (UAM or AF). Numbers correspond to localities in Figure 4 (continued).

8. Washington State (n=31)

Kittitas County: UAM 41846, 41848, 41861-41865, 41870-41872, 41875, 41877, 41878, 41884, 46944, 50883, 50875, 50557, 50579, 50889, 50890, 50890, 50888, 50894, 50901, 50907, 50910; Lewis County: UAM 41672-41675, 41677.

IX. Chapter 2

A Phylogeographic Perspective of the Endemic Red-backed Voles of Southeast Alaska (*Clethrionomys rutilus* and *C. gapperi*)[2]

ABSTRACT

The highly fragmented landscape of southeast Alaska supports 24 endemic mammalian taxa. Among these are two subspecies of red-backed voles that are endemic to islands (*Clethrionomys gapperi wrangeli* and *C. g. solus*) and three endemics that are restricted to the mainland *(C. g. phaeus, C. g. stikinensis*, and *C. rutilus glacialis)*. To assess geographic variation among these taxa, the mitochondrial cytochrome *b* gene (1143 bp) was sequenced from 21 individuals of *C. rutilus* from southeast Alaska, Russia, Finland, and Canada, and from 51 individuals of *C. gapperi* from southeast Alaska, Canada, Washington, Minnesota, Pennsylvania, and North Carolina. *C. rutilus* exhibited low levels of genetic variation, a result consistent with the fossil record, which indicates late Pleistocene colonization of North America by this species. *C. gapperi* formed four distinctive clades in North America, one on the east coast, one on the west coast, a third extending from southeast Alaska to Minnesota, and a southeast Alaska clade that showed genetic introgression from *C. rutilus*. This introgressed clade included individuals south of the Stikine River to Walker Cove. Low, but consistent, levels of genetic variation were found among the subspecies in southeast Alaska, which is consistent with their endemic subspecific designations, with the possible exception of *C. r. glacialis*.

---

[2]Runck, A. M., and J. A. Cook. In prep. A Phylogeographic Perspective of the Endemic Red-backed Voles of Southeast Alaska (*Clethrionomys rutilus* and *C. gapperi*). Canadian Journal of Zoology.

INTRODUCTION

The temperate rain forest of southeast Alaska is naturally fragmented, with over 2,000 islands, extensive icefields, fjords, and six major rivers, all acting as barriers or filters to mammalian dispersal. In addition, southeast Alaska experienced at least 20 major climatic oscillations during the late Pleistocene (Dansgaard et al. 1993; Greenland Ice-core Project Members 1993; Mann and Hamilton 1995), further altering the geographic distribution of taxa. Natural physiographic fragmentation and a dynamic climatological history are thought to be responsible for the relatively high endemism along the North Pacific Coast of North America (Klein 1965; Cook and MacDonald 2001). Isolated from continental North America by the St. Elias and Coast mountains, this complex ecosystem supports 24 described endemic mammalian taxa (MacDonald and Cook 1996).

An emerging view of colonization patterns and phylogeography of mammalian taxa in southeast Alaska is providing insight into the biogeographic history of the region (Cook et al. 2001). Like several other species of mammals (MacDonald and Cook 1996; Parker et al. 1997; Demboski et al. 1999), *Clethrionomys rutilus* (northern red-backed vole), and *C. gapperi* (southern red-backed vole), reach their North American limits of distribution in southeast Alaska. Distinctive biogeographic histories indicate that the post-glacial colonization of these two species into the region occurred by different routes, with deglaciation and the colonization of vegetation affecting their movement into the region. *C. rutilus* is believed to be a Late Wisconsin colonizer of North America from Asia (Gromov and Polyakov 1977). The species is documented from the late Pleistocene

from north of the Cordilleran and Laurentide ice sheets in eastern Beringia (Gromov and Polyakov 1977), and would have colonized southeast Alaska from the North. *C. gapperi* has been dated to the middle Pleistocene of North America (Hibbard et al. 1965; Graham 1976), and was thought to persist in refugia south of the Cordilleran and Laurentide ice sheets.

C. rutilus has a Holarctic distribution, inhabiting northern Europe, Asia, Alaska, and Canada. *C. gapperi* is restricted to North America, inhabiting the forests of the Hudsonian and Canadian life zones of central and southern Canada, northern United States, and has populations extending south, into the Rocky and Appalachian mountain ranges. In southeast Alaska, *C. rutilus* and *C. gapperi* are largely confined to the mainland, only documented from eight nearshore islands. Two subspecies (*C. g. wrangeli* and *C. g. solus*) are endemic to islands, and three subspecies are restricted to the mainland (*C. r. glacialis*, *C. g. stikinensis*, and *C. g. phaeus*; Figure 1). Re-assessment of these taxa with molecular markers provides the framework to more carefully assess the impact of increased anthropogenic disturbances, such as logging, on these endemics in southeast Alaska. These analyses also provide the opportunity to examine the biogeographic history of the region by providing a perspective on the patterns of diversification in the region.

The mitochondrial cytochrome *b* gene was sequenced to elucidate levels of variation of these endemic subspecies along the North Pacific Coast. This study focused on specimens from southeast Alaska and included individuals from other localities to assess variation across their geographic distribution.

MATERIALS AND METHODS

Genomic DNA was extracted from cryogenically preserved tissues (heart, kidney, or liver) using a modified salt extraction protocol (Miller et al.1988). Polymerase chain reaction (PCR) was completed using a Perkin Elmer thermocycler 9600 with primer pairs designed for *Clethrionomys* (Cook et al. in prep; Runck and Cook in prep.). Reactions were carried out under standard protocols (Lessa and Cook 1998) and included negative controls. PCR products were visualized on 1.5% agarose gels and were purified using polyethylene glycol (PEG) precipitation. Purified PCR products were cycle-sequenced using the Taq DyeDeoxy terminator cycle sequencing kit (Perkin Elmer/ABI). Automated sequencing of both heavy and light strands was done on an Applied Biosystems Incorporated 373 DNA sequencer. Sequences were aligned by eye using Sequence Navigator, Version 1.01 (ABI).

To examine relationships among populations of *C. rutilus* in southeast Alaska, complete cytochrome *b* gene sequences were generated from individuals from: southeast Alaska (n=14), interior Alaska (n=1), British Columbia (n=1), Yukon Territory (n=1), Finland (n=2), and Russia (n=2). Complete cytochrome *b* gene sequences from *C. gapperi* included: southeast Alaska (n=43), Minnesota (n=1), North Carolina (n=2), Pennsylvania (n=2), and Washington, (n=1). *Alticola macrotis* (n=2) has been determined to be sister to *C. rutilus* and *C. gapperi* (Conroy and Cook 1999), and was included as an outgroup (Table 1). Phylogenetic analyses of *C. rutilus* and *C. gapperi* were conducted independently.

Phylogenetic analyses were conducted using unweighted maximum parsimony (MP) and maximum likelihood (ML) in the program PAUP*4.0b4a (Swofford 2000). A parsimony search of 1000 trees was performed on both data sets, with all characters having equal weight. The *C. rutilus* data set was bootstrapped with 500 replicates of 1000 randomly generated trees. Bootstrap support was computed on a reduced *C. gapperi* data set (n=34) from which identical sequences were removed. Parameters for maximum likelihood models were estimated and likelihood ratios tests were performed among them using a χ^2 distribution to determine the best model (Sullivan et al. 1999). Maximum likelihood analysis was performed with all taxa included from the *C. rutilus* data set, and was bootstrapped with 100 replicates. A reduced data set of *C. gapperi* was used in maximum likelihood analysis (n=34), in which identical sequences were removed, and was bootstrapped with 50 replicates.

RESULTS

Cytochrome *b* sequences of *C. rutilus* and *C. gapperi*

The complete cytochrome *b* gene of *Clethrionomys* consisted of 1143 base pairs (bp), the same length as other arvicoline rodents (Conroy and Cook 1999; Cook et al. in prep). Expected nucleotide composition biases for mammalian (Irwin 1991) and arvicoline rodents (Conroy and Cook 1999; Cook et al. in prep) were observed. Substitutions were most abundant in third positions and least abundant in the second positions of codons. A deficit of guanine (13%) was observed in comparison to other bases: adenine (30%), cytosine (30%), and thymine (27%).

Phylogeographic analyses of C. rutilus

Phylogenetic analysis of *C. rutilus* using unweighted maximum parsimony produced 51 trees with 207 steps, consistency indexes (C.I.) of 0.778, and retention indexes (R.I.) of 0.854. Of the 1143 characters, 991 characters were constant, 129 characters were parsimony informative, and 23 were uninformative. High bootstrap support (≥ 98%) identified three major clades from Finland, Russia, and Alaska. Genetic divergence between Old World and New World *C. rutilus* did not exceed 3.0% (uncorrected "p"; Table 2). Levels of divergence between individuals from southeast Alaska and interior Alaska were 0 – 0.4%. Levels of divergence within southeast Alaska did not exceed 0.7%, with the greatest level of divergence observed between the endemic *C. r. glacialis* from Excursion Inlet and an individual from Juneau. A lower bootstrap value (80%) supported one clade in southeast Alaska (Figure 2).

Although specimens representing the endemic subspecies *C. r. glacialis* possessed three character state changes that distinguished them from other populations (two third positions transitions and one second position transition; Table 3), bootstrap analysis suggested this was a weak relationship. Overall, there was little support for geographic association with genetic variation.

Maximum likelihood analysis using the HKY85 + Γ likelihood model was chosen because more complex models were not significantly more likely. This model produced 11 best trees. Bootstrap analysis identified the same well-supported relationships as maximum-parsimony (Figure 3).

Phylogeographic analyses of C. gapperi

Unweighted maximum parsimony analysis produced 624 trees (Figure 4) with lengths of 276, C.I.=0.743, and R.I.=0.963. Of the 1143 characters, 946 were constant, 150 were informative, and 47 were uninformative. *C. gapperi* was paraphyletic with respect to *C. rutilus*, and formed two highly divergent (p=8.0%) clades (Cook et al. 2001). One clade consisted of *C. gapperi* from the Stikine River area and *C. rutilus*. The other clade consisted of all other individuals of *C. gapperi*. Analyses of additional molecular and morphological markers are consistent with the hypothesis that paraphyly of *C. gapperi* is the result of post-glacial contact, and subsequent introgression of the mitochondrial genome of *C. rutilus* into *C. gapperi* (Runck and Cook in prep).

High bootstrap support (>98%) defined four major North American clades (east coast, west coast, Alaska, and Minnesota; Figure 4). Genetic divergence of southeast Alaska individuals varied when compared across conspecific populations from Washington (4.19-4.72%), Minnesota (1.13-1.74%), Pennsylvania (5.22-5.97%), and North Carolina (5.77-6.47%; Table 4). The largest intraspecific divergence within southeast Alaska (not including the introgressed *C. gapperi* in the contact zone) was 0.87%. The island endemic populations on Revillagigedo, Wrangell, and Etolin islands were 0.18 – 0.87% divergent from mainland populations. Four clades identified within southeast Alaska (Figure 4) had bootstrap support (>65%) corresponding to the following geographic localities and subspecies: Cleveland Peninsula (*C.g. stikinensis*), Revillagigedo Island (*C. g. solus*), Wrangell and Etolin islands (*C. g. wrangeli*), and the southern southeast Alaska mainland (*C. g. phaeus)*. Two synapomorphies (a second and

third position transition; Table 5) distinguished *C. g. wrangeli* on Wrangell and Etolin islands from mainland populations, and individuals south of Walker Cove to Rudyerd Bay formed a clade diagnosed by one synapomorphy (a second position transition). The subspecies *solus* found on Revillagigedo Island possessed more variation than *wrangeli* individuals, and formed three clades.

Maximum likelihood using HKY85 + Γ was chosen because more complex models were not significantly more likely. Eight trees were generated, and bootstrap analysis supported the four major North American clades and four southeast Alaskan clades (Figure 5).

DISCUSSION

Geographic variation and colonization in southeast Alaska

Lack of geographic structure within Alaska and low levels of genetic divergence in relation to conspecific Old World individuals suggest a recent trans-Beringian colonization of *C. rutilus*, a pattern consistent with the fossil record (Gromov and Polyakov 1977; Cook et al. in prep). Post-glacial colonizers are characterized by reduced levels of genetic diversity when compared to conspecific southern populations (Hayes and Harrison 1992; Hewitt 1996; Hewitt 1999; Pamilo and Savolainen 1999; but see Tegelström and Jäärola 1989; Fedorov et al. 1999). Other late Beringian colonizers, such as the tundra vole (*Microtus oeconomus*; Lance and Cook 1998) exhibit lack of geographic structure and low levels of genetic divergence among their populations. Minimal levels of genetic differentiation were observed between individuals from southeast and interior Alaska, suggesting a recent post-glacial colonization of *C. rutilus*

into southeast Alaska. Persisting north of the Laurentide and Cordilleran ice sheets, *C. rutilus* would have colonized southeast Alaska as the ice sheets receded. These routes from Beringia into southeast Alaska were believed to be glaciated longer than routes from the south (Mandryk 1996), delaying the southward colonization of the North Pacific Coast of North America (Lance and Cook 1998; Fleming and Cook in prep).

In contrast, *C. gapperi* apparently persisted in refugia south of the Cordilleran and Laurentide ice sheets during the Late Wisconsin (MacPherson 1965). High levels of genetic divergence between the eastern (east of the Appalachians) and western (west of the Cascades) clades are consistent with the hypothesis that at least two southern refugia existed south of the major continental glaciers. East and west patterns of divergence have been observed in other forest-associated mammal taxa such as American marten (*Martes americana*; Stone et al. submitted) and black bear (*Ursus americana*; Wooding and Ward 1997; Stone and Cook 2000). However, unlike these taxa, *C. gapperi* in southeast Alaska are not represented by two distinctive clades. Populations of *C. gapperi* in southeast Alaska are divergent ($p>4.0\%$) from populations on the east and west coasts, and are most closely related to individuals from Minnesota ($p=1.1$). Levels of genetic divergence among southeast Alaska *C. gapperi* were minimal, but the limited geographic structure of genetic variation suggested an earlier colonization of southeast Alaska than *C. rutilus*.

The lack of red-backed voles on islands in southeast Alaska (Hall 1981) is probably related to their poor ability to disperse over water (Grant 1970). Colonization of *C. gapperi* onto nearshore islands may have occurred when ocean levels were lower, and distances to islands were shorter, around 10,000 ybp (McKenzie and Goldthwait

1971; Mobley 1988). Dispersal onto islands may have been mediated by rafting from the mainland. Grant (1970) hypothesized that red-backed voles may be competitively excluded by resident species of *Microtus*; therefore, they may be poor island colonizers if populations of *Microtus* are already present. An earlier arrival of *Microtus longicaudus* into the region may have limited the colonization of only eight nearshore islands in southeast Alaska by *C. rutilus* and *C. gapperi*. *M. longicaudus,* and to a lesser extent *M. oeconomus* and *M. pennsylvanicus,* inhabit many islands of the Alexander Archipelago (Hall 1981).

Subspecific taxonomy and phylogeography of Clethrionomys in southeast Alaska

The endemic red-backed voles in southeast Alaska were originally described from differences in morphological characters such as pelage color, skull size, and tail length. *C. r. glacialis* was diagnosed from other Alaskan populations of *C. rutilus* by its larger size and darker pelage (Orr 1945; Rausch 1950). *C. g. phaeus* is distinguished from the other mainland endemic, *C. g. stikinensis*, by a larger cranium and less inflated auditory bullae (Swarth 1911; Hall and Cockrum 1952). The island endemic *C. g. solus* is distinguished from the other island population, *C. g. wrangeli* by a shallower skull and a brighter dorsal stripe (Bailey 1897; Hall and Cockrum 1952). Because the descriptions of these endemic populations were based on specimens from limited locations, their ranges were probably extrapolated in respect to topographic features and geographic barriers, with rivers, mountain ranges, or ice fields demarcating their populations.

Genetic diversity was assessed within and among the five endemic southeast Alaskan populations. Genetic diversity within *C. rutilus* was not associated with

geographic location of populations in Alaska. Identical haplotypes were found in Interior and southeast Alaskan populations of *C. rutilus.* This lack of genetic structure is probably due to the relatively recent colonization of *C. rutilus* of North America. Unlike *C. rutilus*, *C. gapperi* did not possess shared haplotypes among distinctive populations suggesting that recent gene flow has been limited. Though relatively low levels of genetic divergence were observed among these populations, phylogeographic patterns among populations of *C. gapperi* corresponded to their subspecific taxonomy and geographic barriers.

Subspecies of *C. gapperi* in this region appear to be neoendemics that are characterized by low, but consistent, levels of genetic divergence and minimal geographic genetic structure. Within subspecies, additional genetic structure corresponding to the complex landscape of southeast Alaska was observed. For example, in the southern part of southeast Alaska, the subspecies *C. g. phaeus* formed two clades. This area is highly fragmented, surrounded by glaciers and mountains, and is separated from continental North America by the Portland Canal. The minimal genetic variation within these clades is probably due to recent founder events, population bottlenecks, or limited contemporary gene flow in these evolutionarily young populations.

Low levels of genetic variation among individuals of *C. g. wrangeli* on Wrangell and Etolin islands (8 out of 10 individuals shared the same haplotype) may be related to recent founder events or bottlenecks. If colonization proceeded from the mainland to Wrangell to Etolin Island, a small colonizing population on Wrangell Island and subsequent bottlenecks could result in low genetic diversity and identical haplotypes on

these islands. Revillagigedo Island populations (*C. g. solus*), in contrast, were found to be more variable, a difference that may relate to either the greater effect of population fluctuations on reducing genetic variation on smaller islands (e. g. Wrangell Island), or higher rates of exchange between the mainland and Revillagigedo Island.

Populations of *C. g. stikinensis* from the southern part of the Cleveland Peninsula formed a strongly supported clade. These populations appear to be isolated from the rest of the mainland populations by adjacent mainland populations of *C. gapperi* which have the mitochondrial genome sequence of *C. rutilus* (Runck and Cook in prep.). Analyses of three independent markers showed that the mitochondrial genome of *C. rutilus* has introgressed into all *C. gapperi* examined from the Stikine River area south to Hut Point. Because the '*rutilus*' haplotype was not found in any individuals of *C. gapperi* south of Tyee and Reflection Lake on the Cleveland Peninsula (Runck and Cook in prep.), it appears that gene flow has been restricted between the mainland and Cleveland Peninsula. However, this study assessed only a maternally inherited marker, which will reflect female mediated exchange. Additional independent markers need to be examined to test this hypothesis that the Cleveland Peninsula populations are isolated from other regions in southeast Alaska.

The use of a phylogenetic perspective to address the status of endemics is now central to conservation biology (Firestone et al. 1999; Moritz 1999). By diagnosing potentially distinct populations in terms of evolutionary significant units (ESU), we can begin to understand the role of historical separation in differentiation and identify populations with distinct evolutionarily potential (Waples 1991; Moritz 1994). Recently

Crandall et al. (2000) argued that adaptive significance also should be demonstrated for distinctive ESU's.

While it is not possible at this time to demonstrate adaptive differences among these populations, phenotypic differences that distinguish these subspecies may be adaptive. Because genetic divergence was concordant with subspecies designations (which were originally described from phenotypic differences), a relationship between phenotypic and genetic differentiation exists in this region. The lack of shared haplotypes among populations and the discovery that phylogenetic divergence that is largely concordant with geographic barriers suggest low levels of gene flow. This study of one marker lays the foundation for analyses of additional independent markers that should be used to further test these findings.

CONCLUSIONS

The two species of red-backed voles in southeast Alaska have distinct biogeographic histories. *C. rutilus* exhibited minimal genetic divergence and lacked geographic differentiation across populations in Alaska. Low genetic variation is consistent with the hypothesis that this species recently colonized North America from Asia. In contrast, *C. gapperi* was found to have three strongly supported and divergent North American clades that appeared to have persisted in more than one southern refugium during the Wisconsin glaciation. Geographically structured genetic variation in southeast Alaska is consistent with the idea that *C. gapperi* colonized the region prior to *C. rutilus.*

Because both species apparently did not colonize southeast Alaska until after the retreat of the major ice sheets about 10,000 ybp, overall genetic differentiation within these species in this region was minimal. Low, but consistent, levels of genetic divergence were observed in *C. gapperi* and corresponded to the subspecific designations of *C. g. phaeus*, *C. g. stikinensis*, *C. g. wrangeli* and *C. g. solus.* Additional genetic structure, although minimal, was concordant with major geographic barriers in the region. Because the patterns of genetic diversity were closely associated with subspecific taxonomy, with the possible exception of *C. r. glacialis*, additional sampling of populations and genes should further investigate the taxonomic status of these endemics.

ACKNOWLEDGMENTS

Brandy Jacobsen and Tom LeCroy assisted with laboratory support. John Bender II, Richard Runck, Leigh Dickey, Howie Chen, Allison Bidlack, Tom Seaton, Steve MacDonald, Doug Clark, and Laura Elze provided excellent help with fieldwork. Special thanks to Cole Crocker-Bedford, Mike Brown, Ed Grossman, Joe Spicanni, Peg Robertson, Lynn Atkins, and Scott Posner for assistance with logistics while collecting in southeast Alaska. The Museum of Southwestern Biology provided *C. gapperi* tissue samples from the western United States. Brian Arbogast provided *C. gapperi* tissue from the eastern United States. Beverly Johnson conducted a pilot project that led to this expanded analysis. This work was funded by the US Fish and Wildlife Service, USDA Forest Service, and the National Science Foundation.

LITERATURE CITED

Bailey, V. 1897. *Evotomys wrangeli*. Proc. Biol. Soc. Wash. **11**:120.

Conroy, C. J., and Cook., J. A. 1999. MtDNA evidence for repeated pulses of speciation within arvicoline and murid rodents. Journal of Mammalian Evolution, **6**:221-245.

Cook, J. A., Bidlack, A. L., Conroy, C. J., Demboski, J. R., Fleming, M. A., Runck, A. M., Stone, K. D., and MacDonald, S. O. 2001. A phylogeographic perspective on endemism in the Alexander Archipelago of the North Pacific. Biol. Conserv. **97**:215-227.

Cook, J. A., and MacDonald, S. O. 2001. Should endemism be a focus of conservation efforts along the North Pacific Coast of North America? Biol. Conserv. **97**:207-213.

Cook, J. A., Runck, A. M., and Conroy, C. J. in preparation. Historical biogeography at the crossroads of the northern continents: Molecular phylogenetics of red-backed voles (Rodentia: Arvicolidae).

Crandall, K. A., Binida-Emonds, O. R. P., Mace, G. M., and Wayne, R. K. 2000. Considering evolutionary processes in conservation biology. Trends Ecol. Evol. **15**(7):290-295.

Demboski, J. R., Stone, K. D., and Cook, J. A. 1999. Further perspectives on the Haida Gwaii glacial refugium. Evolution, **53**:2008-2012.

Dansgaard, W., Johnsen, S. J., Clusen, H. B., Dahl-Jensen, D., Gundestrup, N. S., Hammer, G. U., Hvidberg, C. S., Steffensen, J. P., Sveinbjornsdottir, E. E., Jouzel, J.,

and Bond, G. 1993. Evidence for general instability of past climate from a 250-kyr ice-core. Nature, **364**:218-220.

Fedorov, V., Goropashnaya, A., Jarrell, G. H., and Fredga, K. 1999. Phylogeographic structure and mitochondrial DNA variation in true lemmings (*Lemmus*) from the Eurasian Arctic. Biol. J. Linn. Soc. **66**:357-371.

Firestone, K. B., Elphinstone, M. S., Sherwin, W. B., and Houlden, B. A. 1999. Phylogeographic population structure of tiger quolls *Dasyurus maculatus* (Dasyuridae: Marsupialia), an endangered carnivorous marsupial. Mol. Ecol. **8**:1613-1625.

Fleming, M. A., and J. A. Cook. in preparation. Phylogeography of endemic ermine (*Mustela erminea*) in southeast Alaska.

Graham, R. W. 1976. Late Wisconsin mammalian faunas and environmental gradient of the eastern United States. Paleobiology, **2**:343-350.

Grant, P. R. 1970. Colonization of islands by ecologically dissimilar species of mammals. Can. J. Zool. **48**:545-553.

Greenland Ice-core Project Members. 1993. Climate instability during the last interglacial period recorded in the GRIP ice core. Nature, **364**:203-207.

Gromov, I. M., and Polyakov, I. Y. 1977. Voles (Microtinae), Fauna of the USSR, Mammals, Volume II, No. 8. Smithsonian Institution Libraries and the National Science Foundation, Washington, D. C.

Hall, E. R. 1981. The Mammals of North America. Second ed. John Wiley and Sons, New York **1**:1-600.

Hall, E. R., and Cockrum, E. L. 1952. Comments on the taxonomy and geographic

distribution of North American microtines. University of Kansas Publications Museum of Natural History, **5**(23):293-312.

Hayes, J. P., and Harrison, R. G. 1992. Variation in mitochondrial DNA and the biogeographic history of woodrats (*Neotoma*) of the Eastern United States. Syst. Biol. **41**:331-344.

Hewitt, G. M. 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. Biol. J. Linn. Soc. **58**:247-276.

Hewitt, G. M. 1999. Post-glacial re-colonization of European biota. Biol. J. Linn. Soc. **68**:87-112.

Hibbard, C. W., Ray, C. E., Savage, D. E., Taylor, D. W., and Guilday, J. E. 1965. Quaternary mammals of North America. *In* The Quaternary of the United States. *Edited by* H. E. Wright, Jr., and D. G. Frey. Princeton University Press, Princeton, New Jersey pp.509-525.

Irwin, D. M., Kocher, T. D., and Wilson, A. C. 1991. Evolution of the cytochrome *b* gene of mammals. J. Mol. Evol. **32**:128-144.

Klein, D. R. 1965. Postglacial distribution patterns of mammals in the southern coastal regions of Alaska. Arctic, **18**:7-20.

Lance, E. W., and Cook, J. A. 1998. Biogeography of tundra voles (*Microtus oeconomus*) of Beringia and the southern coast of Alaska. J. Mammal. **79**:53-65.

Lessa, E. P., and Cook, J. A. 1998. The molecular phylogenetics of tuco-tucos (genus *Ctenomys*, Rodentia: Octodontidae) suggests an early burst of speciation. Mol. Phylogenet. Evol. **9**:88-99.

MacDonald, S. O., and Cook, J. A. 1996. The land mammal fauna of southeast Alaska. Can. Field-Nat. **110**:571-598.

MacPherson, A. H. 1965. The origin of diversity in mammals of the Canadian arctic tundra. Syst. Zool. **14**:153-173.

Mandryk, C. A. S. 1996. Late Wisconsin deglaciation of Alberta: processes and paleogeography. Quaternary International, **32**:79-85.

Mann, D. H., and Hamilton, T. D. 1995. Late Pleistocene and Holocene paleoenvironments of the North Pacific coast. Quaternary Science Review, **14**:449-471.

McKenzie, G. D., and Goldthwait, R. G. 1971. Glacial history of the last eleven thousand years in Adams Inlet, southeastern Alaska. Geological Society of America Bulletin, **82**:1767- 1782.

Miller, S. A., Dykes, D. D., and Polesky, H. F. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acid Res. **16**:1215.

Mobley, C. M. 1988. Holocene sea levels in southeast Alaska: preliminary results. Arctic, **41**:261-266.

Moritz, C. 1994. Defining 'evolutionary significant units' for conservation. Trends Ecol. Evol. **9**:373-375.

Moritz, C. 1999. Conservation units and translocations: strategies for conserving evolutionary processes. Hereditas, **130**(3):217-228.

Orr, R. T. 1945. A study of the *Clethrionomys dawsoni* group of red-backed voles.

J. Mammal. **26**:67-74.

Pamilo, P., and Savolainen, O. 1999. Post-glacial colonization, drift, local selection and conservation value of populations: a northern perspective. Hereditas, **130**:229-238.

Parker, D. I., Lawhead, B. E., and Cook, J. A. 1997. Distributional limits of bats in Alaska. Arctic, **50**:1283-1292.

Rausch, R. J. 1950. *Clethrionomys rutilus glacialis*. Journal Washington Academy of Science, **40**:135.

Runck, A. M., and Cook, J. A. in preparation. Molecular and morphological analysis of a contact zone in red-backed voles (Genus *Clethrionomys*).

Stone, K. D., and Cook, J. A. 2000. Phylogeography of black bears (*Ursus americanus*) of the Pacific Northwest. Can. J. Zool. **78**:1218-1223.

Stone, K. D., Flynn, R. W., and Cook, J. A. submitted. Post-glacial colonization of northwestern North America by the forest associated American marten (*Martes americana*). Mol. Ecol.

Swarth H. S. 1911. Birds and mammals of the 1909 Alexander Alaska Expedition. University of California Publications in Zoology, **7**(2):9-172.

Sullivan, J., D. Swofford, L., and Naylor, G. J. P. 1999. The effect of taxon sampling on estimating rate heterogeneity parameters of maximum-likelihood models. Mol. Biol. Evol. **16**(10):1347-1356.

Swofford, D. L. 2000. PAUP*: Phylogenetic Analysis Using Parsimony (*and other methods). Sinauer Associates, Sunderland, Massachusetts.

Tegelström, H., and Jaarola, M. 1989. Genetic divergence in mitochondrial DNA

between the wood mouse (*Apodemus sylvaticus*) and the yellow necked mouse (*A. flavicollis*). Hereditas, **111**:49-60.

Waples, R. S. 1991. Pacific salmon, *Oncorhynchus* spp., and the definition of "species" under the Endangered Species Act. Mar. Fish. Rev. **53**:11-22.

Wooding, S., and Ward, R. 1997. Phylogeography and Pleistocene evolution in the North American black bear. Mol. Biol. Evol. **14**:1096-1105.

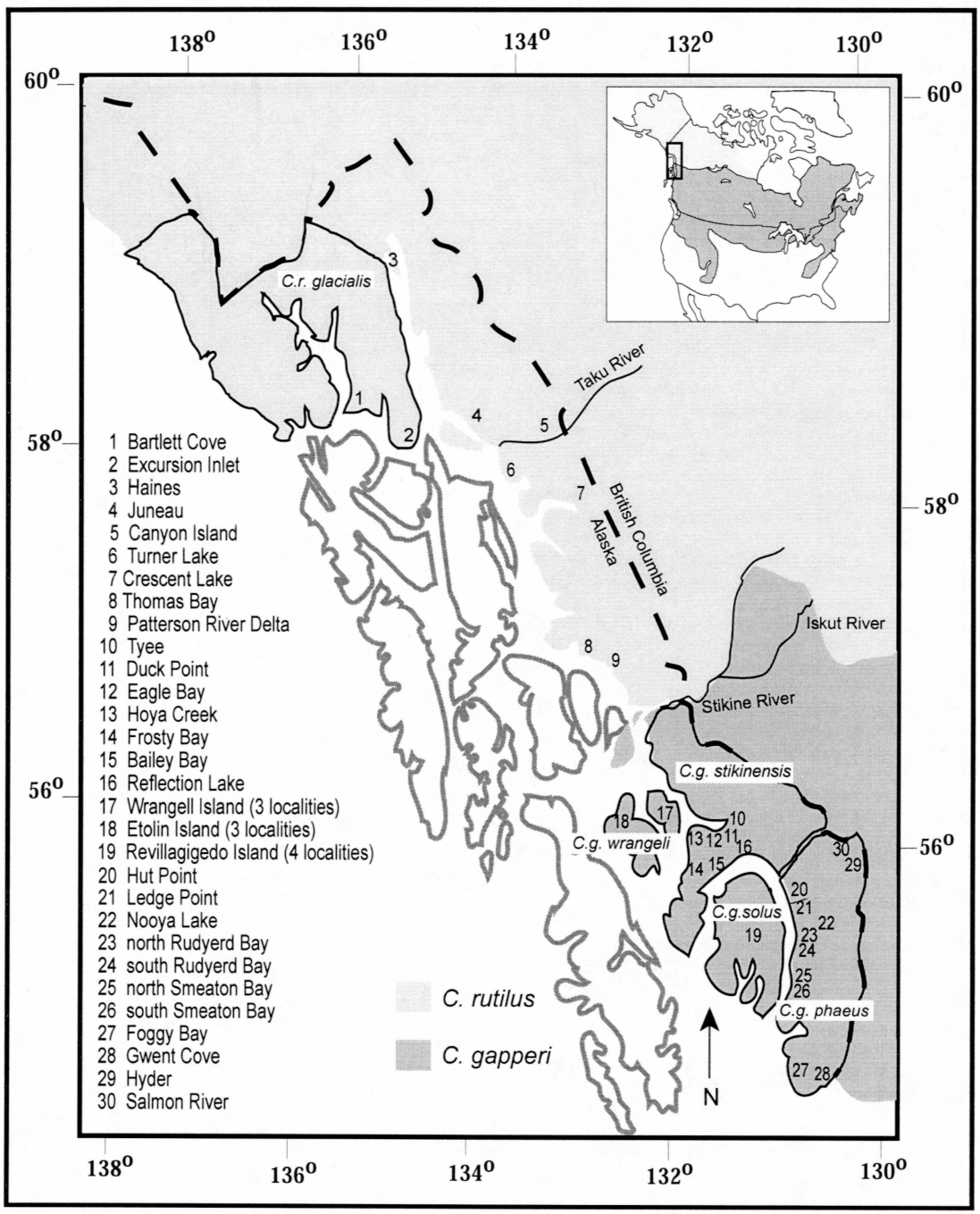


Figure 1. Distribution and sampling localities of *Clethrionomys rutilus* and *C. gapperi* and endemic subspecies in southeast Alaska.

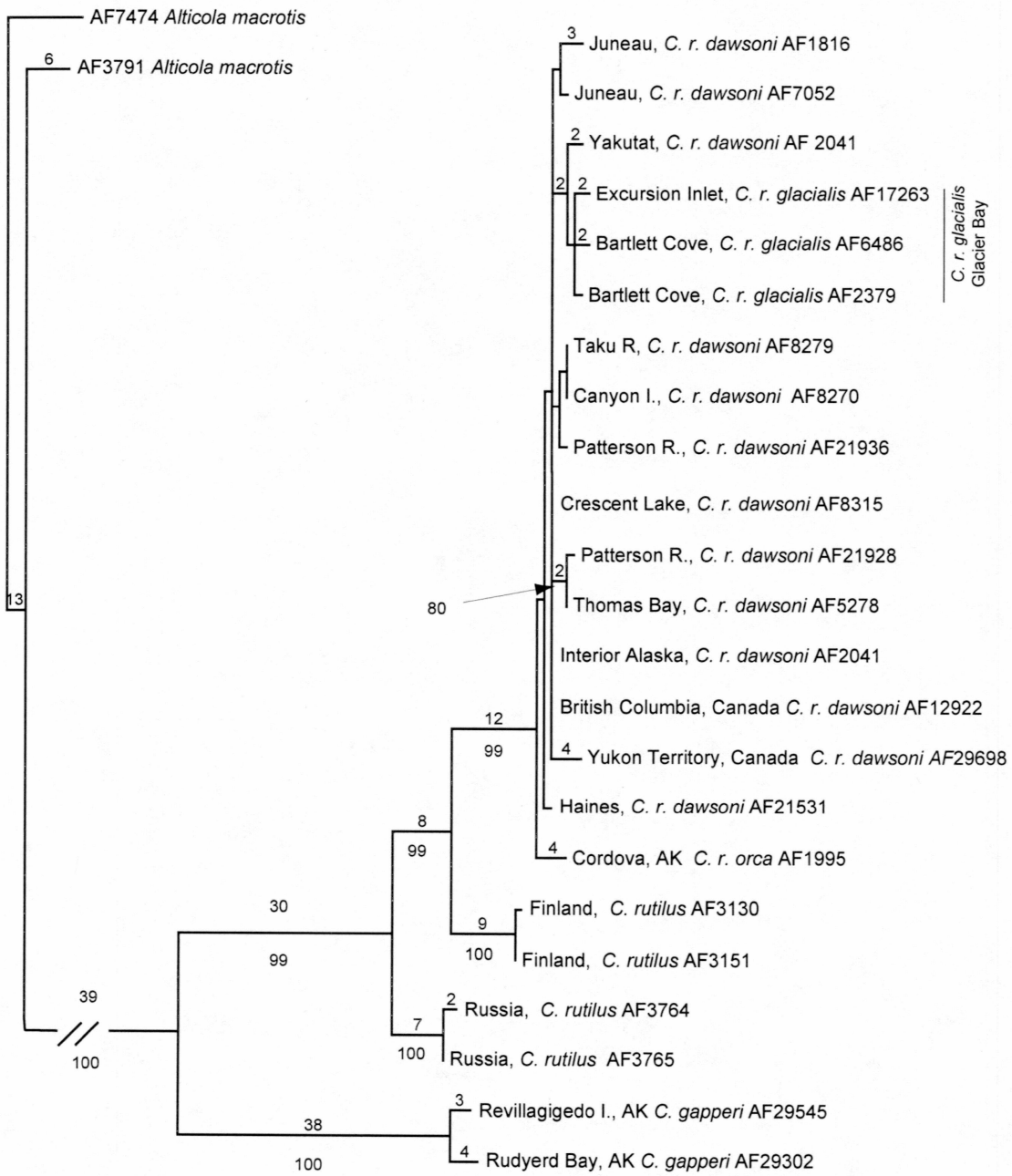


Figure 2. Maximum parsimony (unweighted) tree constructed from complete cytochrome *b* sequences (1143 bp) for *C. rutilus*. This is one of 51 most parsimonious trees (207 steps). Branch lengths >1 are shown above the branches and bootstrap support greater than 65% (500 replicates of 1000 randomly generated trees) are indicated below the branches. C.I. =0.778 and R.I. =0.854.

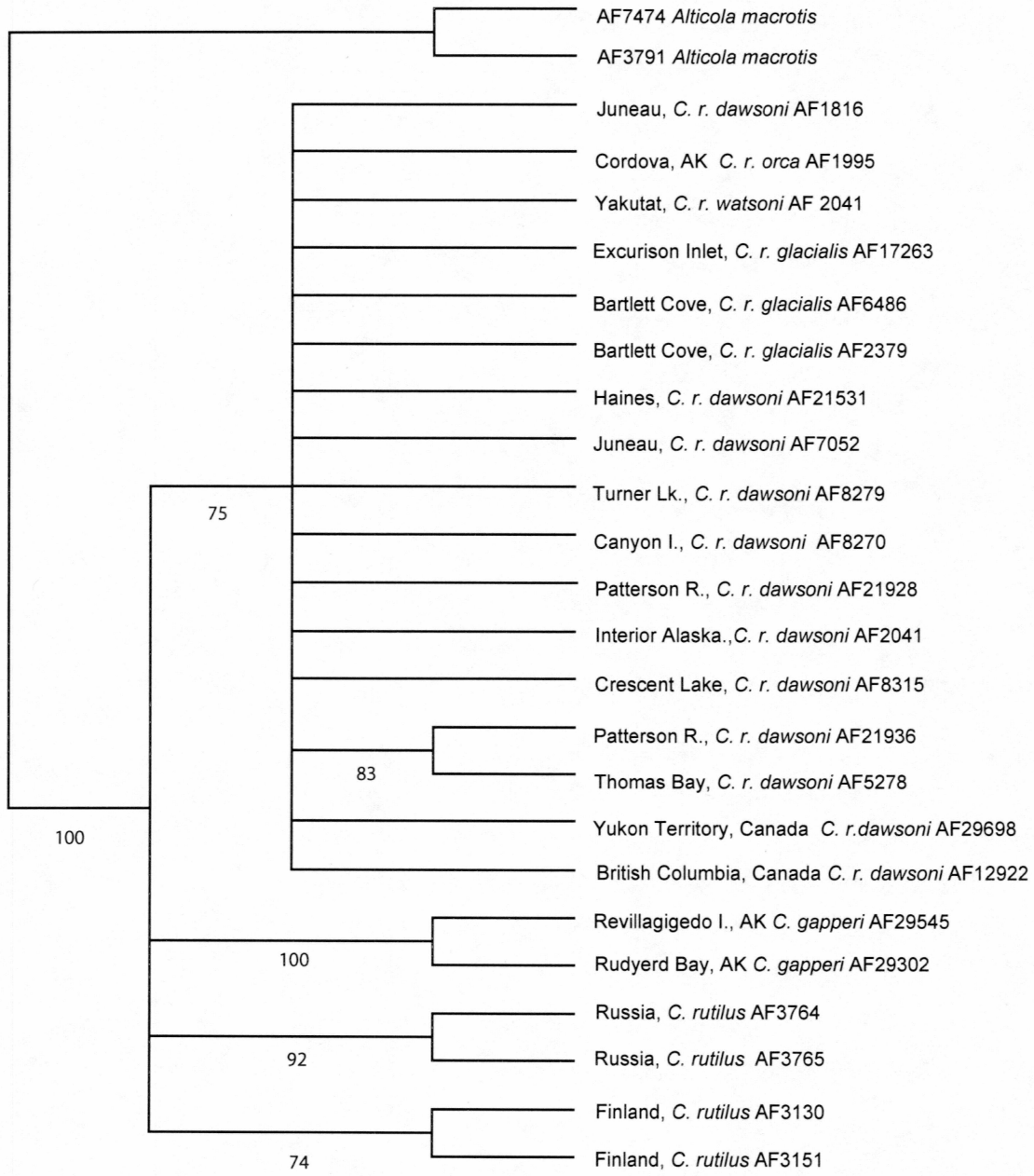


Figure 3. Bootstrap maximum-likelihood using HKY85 + gamma model constructed from complete cytochrome *b* for *C. rutilus*. Bootstrap support >65% are indicated below the branches (100 replicates of 1000 randomly generated trees).

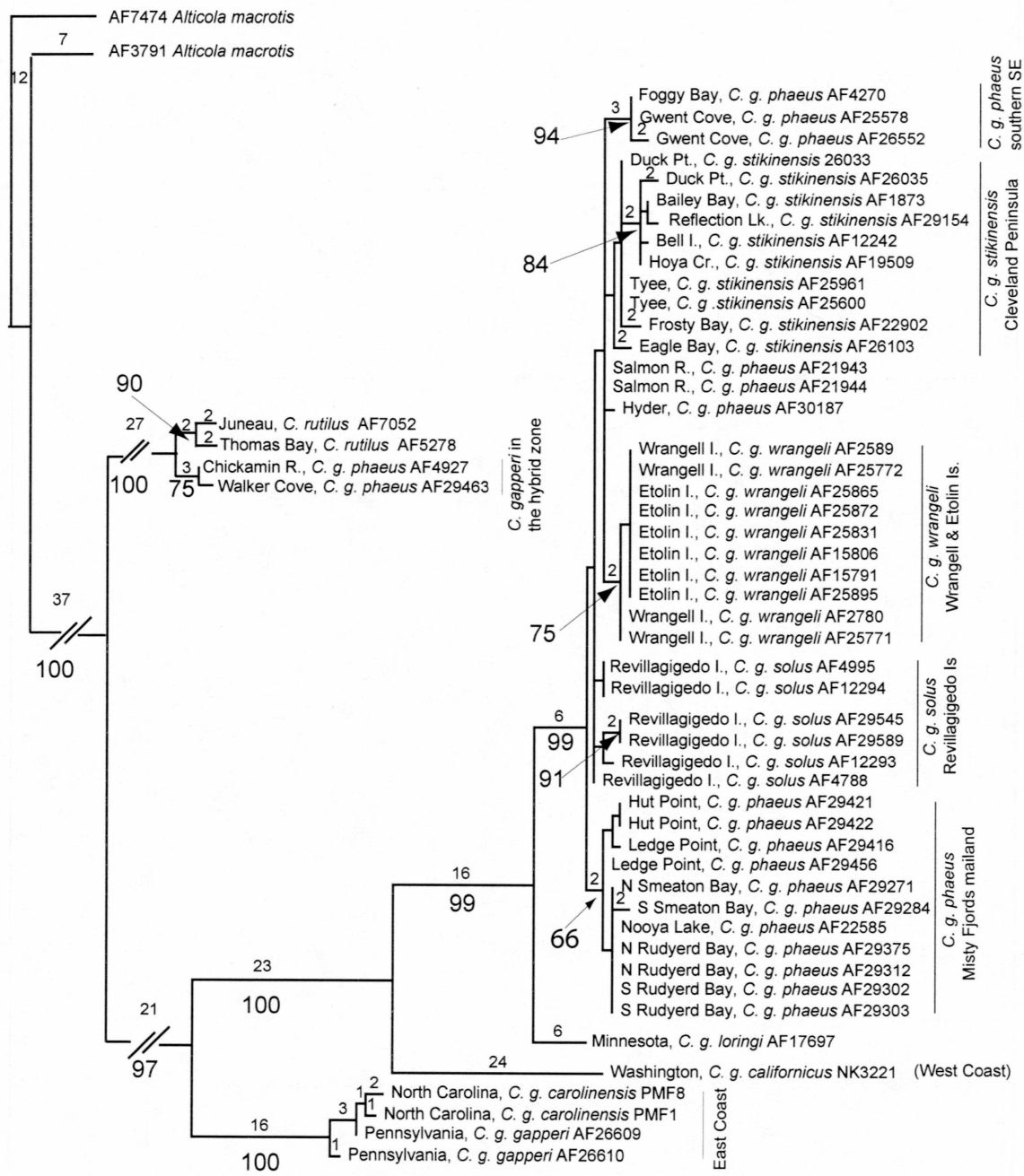


Figure 4. Maximum parsimony (unweighted) tree constructed from complete cytochrome *b* sequences (1143 bp) for *C. gapperi*. This is one of 624 most parsimonious trees (276 steps). Branch lengths > 1 are shown above the branches and bootstrap support >65% (500 replicates of 1000 randomly generated trees) are indicated below the branches. C.I. =0.743 and R. I. =0.963.

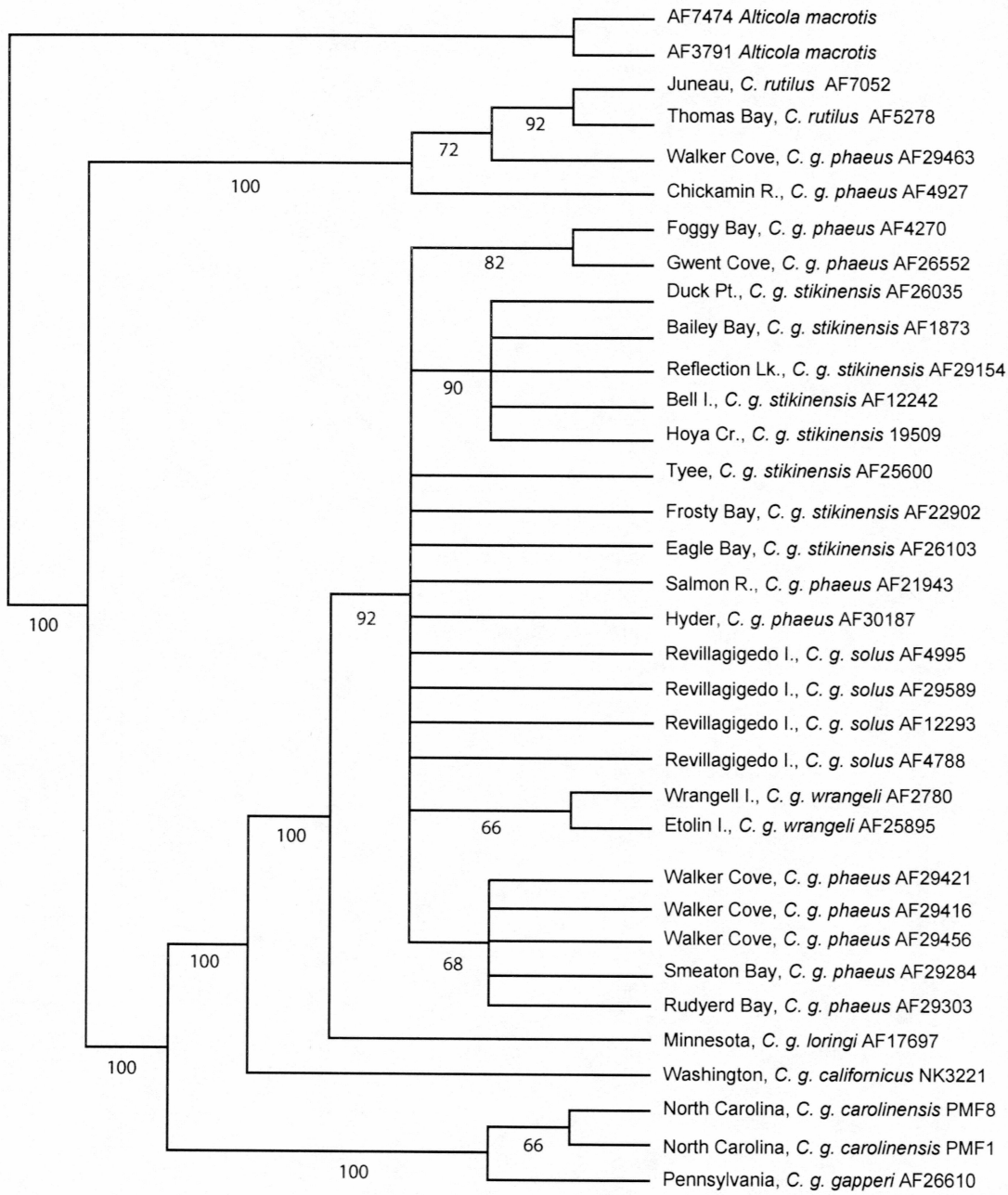


Figure 5. Bootstrap maximum-likelihood using HKY85 + gamma model constructed from complete cytochrome *b* sequences for *C. gapperi*. Bootstrap support>65% are indicated below the branches (50 replicates of 1000 randomly generated trees).

Table 1. Specimens, localities, and voucher numbers.

Taxa		Location	Source
Outgroup			
Alticola macrotis	(n=2)	Russia	AF3791; AF7474
Ingroup			
Clethrionomys			
rutilus	(n=2)	Russia	AF3764; AF3765
rutilus	(n=2)	Finland	AF3130; AF3151
rutilus watsoni		Alaska: Yakutat	AF2041
rutilus orca		Alaska: Cordova	AF1995
rutilus dawsoni		Alaska: Denali Nat. Park	AF2041
rutilus dawsoni		Canada: Yukon Territory	AF29698
rutilus dawsoni		Canada: British Columbia	AF12922
rutilus dawsoni	(n=2)	Alaska: Juneau	AF1816; AF7052
rutilus dawsoni		Alaska: Haines	AF21531
rutilus dawsoni		Alaska: Canyon Island	AF8270
rutilus dawsoni		Alaska: Turner Lake	AF8279
rutilus dawsoni		Alaska: Crescent Lake	AF8315
rutilus dawsoni		Alaska: Thomas Bay	AF5278

Table 1. Specimens, localities, and voucher numbers (continued).

Taxa		Location	Source
rutilus dawsoni	(n=2)	Alaska: Patterson River	AF21928; AF21936
rutilus glacialis		Alaska: Excursion Inlet	AF17263
rutilus glacialis	(n=2)	Alaska: Bartlett Cove	AF6486; AF6508
gapperi carolinensis	(n=2)	North Carolina	[2]PMF1; PMF8
gapperi gapperi	(n=2)	Pennsylvania	AF22609; AF22610
gapperi loringi		Minnesota	AF17697
gapperi californicus		Washington	[3]NK3221
gapperi phaeus		Alaska: Chickamin River	AF4927
gapperi phaeus		Alaska: Foggy Bay	AF4270
gapperi phaeus	(n=2)	Alaska: Gwent Cove	AF26578; AF26552
gapperi phaeus	(n=2)	Alaska: Salmon River	AF21943; AF21944
gapperi phaeus		Alaska: Hyder	AF30187
gapperi phaeus	(n=3)	Alaska: Hut Point	AF29421; AF29422; AF29463
gapperi phaeus	(n=2)	Alaska: Ledge Point	AF29416; AF29456
gapperi phaeus		Alaska: Nooya Lake	AF22585
gapperi phaeus	(n=2)	Alaska: N Rudyerd Bay	AF29312; AF29375
gapperi phaeus	(n=2)	Alaska: S Rudyerd Bay	AF29302; AF29303
gapperi phaeus	(n=2)	Alaska: Smeaton Bay	AF29271; AF29284

Table 1. Specimens, localities, and voucher numbers (continued).

gapperi solus	(n=6)	Alaska: Revillagigedo Island	AF4995; AF4788; AF29545; AF29589; AF12293; AF12294
gapperi stikinensis	(n=2)	Alaska: Tyee	AF25600; AF25961
gapperi stikinensis	(n=2)	Alaska: Duck Point	AF26033; AF26035
gapperi stikinensis		Alaska: Bailey Bay	AF1873
gapperi stikinensis		Alaska: Reflection Lake	AF29154
gapperi stikinensis		Alaska: Bell Island	AF12242
gapperi stikinensis		Alaska: Hoya Creek	AF19509
gapperi stikinensis		Alaska: Frosty Bay	AF22902
gapperi stikinensis		Alaska: Eagle Bay	AF26103
gapperi wrangeli	(n=4)	Alaska: Wrangell Island	AF2589; AF2780; AF25771; AF25772
gapperi wrangeli	(n=6)	Alaska: Etolin Island	AF15791; AF15806; AF25831; AF25865; AF25872; AF25895

[1]AF – Alaska Frozen Tissue Collection, University of Alaska Museum.

[2]PMF- Brian Arbogast, University of Washington.

[3]NK- Museum of Southwestern Biology.

Table 2. Uncorrected pair-wise distances (p) for *Clethrionomys rutilus*.

	1	2	3	4	5	6	7	8	9	10
1 *C. r. dawsoni* Denali										
2 *C. r. orca* Cordova	0.0052									
3 *C. r. dawsoni* Yakutat	0.0034	0.0069								
4 *C. r. dawsoni* Juneau	0.0034	0.0087	0.0069							
5 *C. r. dawsoni* Juneau	0.0017	0.0069	0.0052	0.0034						
6 *C. r. glacialis* Bartlett Cove	0.0043	0.0078	0.0043	0.0061	0.0043					
7 *C. r. glacialis* Bartlett Cove	0.0034	0.0069	0.0034	0.0069	0.0052	0.0026				
8 *C. r. glacialis* Excursion Inlet	0.0043	0.0061	0.0043	0.0078	0.0061	0.0034	0.0026			
9 *C. r. dawsoni* Haines	0.0017	0.0052	0.0052	0.0052	0.0034	0.0061	0.0052	0.0061		
10 *C. r. dawsoni* Canyon Is.	0.0017	0.0069	0.0052	0.0052	0.0034	0.0061	0.0052	0.0061	0.0034	
11 *C. r. dawsoni* Turner Lk.	0.0017	0.0070	0.0052	0.0052	0.0035	0.0061	0.0052	0.0061	0.0035	0
12 *C. r. dawsoni* Crescent Lk.	0	0.0052	0.0034	0.0034	0.0017	0.0043	0.0034	0.0043	0.0017	0.0017
13 *C. r. dawsoni* Thomas Bay	0.0017	0.0069	0.0052	0.0052	0.0034	0.0043	0.0034	0.0043	0.0034	0.0034
14 *C. r. dawsoni* Patterson R.	0.0026	0.0078	0.0061	0.0061	0.0043	0.0052	0.0043	0.0052	0.0043	0.0043
15 *C. r. dawsoni* Patterson R.	0.0017	0.0069	0.0052	0.0052	0.0034	0.0061	0.0052	0.0061	0.0034	0.0017
16 *C. r. dawsoni* B. C.	0	0.0052	0.0035	0.0035	0.0017	0.0043	0.0035	0.0043	0.0017	0.0017
17 *C. r. dawsoni* Y. T.	0.0034	0.0087	0.0069	0.0069	0.0052	0.0078	0.0069	0.0078	0.0052	0.0052
18 *C. rutilus* Russia	0.0271	0.0288	0.0288	0.0306	0.0288	0.0297	0.0288	0.0280	0.0271	0.0271

Table 2. Uncorrected pair-wise distances (p) for *Clethrionomys rutilus* (continued).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
19 *C. rutilus* Russia	0.0253	0.0271	0.0271	0.0288	0.0271	0.0280	0.0271	0.0265	0.0253	0.0253
20 *C. rutilus* Finland	0.0210	0.0227	0.0227	0.0245	0.0227	0.0218	0.0210	0.0201	0.0210	0.0210
21 *C. rutilus* Finland	0.0201	0.0218	0.0218	0.0236	0.0218	0.0227	0.0218	0.0210	0.0201	0.0201

	11	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
12 *C. r. dawsoni* Crescent Lk.	0.0017									
13 *C. r. dawsoni* Thomas Bay	0.0035	0.0017								
14 *C. r. dawsoni* Patterson R.	0.0043	0.0026	0.0008							
15 *C. r. dawsoni* Patterson R.	0.0017	0.0017	0.0034	0.0043						
16 *C. r. dawsoni* B. C.	0.0017	0	0.0017	0.0026	0.0017					
17 *C. r. dawsoni* Y. T.	0.0052	0.0034	0.0052	0.0061	0.0052	0.0035				
18 *C. rutilus* Russia	0.0272	0.0271	0.0288	0.0288	0.0288	0.0280	0.0253			
19 *C. rutilus* Russia	0.0254	0.0253	0.0271	0.0271	0.0271	0.0262	0.0236	0.0017		
20 *C. rutilus* Finland	0.0202	0.0210	0.0210	0.0210	0.0227	0.0218	0.0227	0.0236	0.0218	
21 *C. rutilus* Finland	0.0193	0.0201	0.0218	0.0218	0.0218	0.0210	0.0218	0.0227	0.0210	0.0087

RASMUSON LIBRARY
UNIVERSITY OF ALASKA-FAIRBANKS

Table 3. Polymorphic cytochrome *b* sites in North American *Clethrionomys rutilus*.

Subspecies and Locality	Nucleotide Position																													
	6	1	2	2	2	3	3	4	4	5	5	5	5	6	6	6	6	6	7	7	7	8	8	8	9	9	1	1		
	8	6	7	7	8	3	8	1	4	3	4	5	5	0	1	8	9	9	0	1	9	4	1	7	6	7	0	1		
		7	2	2	0	4	0	8	7	0	5	8	6	2	4	0	3	8	0	5	3	1	7	8	2	2	0	3		
C. r. dawsoni Denali	A	G	A	T	T	T	T	G	T	C	A	G	T	A	G	C	G	G	G	T	G	A	G	G	C	G	C	A		
C. r. orca Cordova	.	A	.	.	.	C	.	.	.	.	G	.	.	.	A	.	.	A	.	.	.	.	.	.	.	.	T	.		
C. r. dawsoni Yakutat	.	.	.	A	.	.	.	.	.	.	.	A	.	.	.	.	.	.	A	.	.	.	.	.	.	.	T	.		
C. r. dawsoni Juneau	G	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	A	.	.	.	.		
C. r. dawsoni Juneau	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	A	.	.	.	.		
C. r. glacialis Bartlett Cove	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	A	C	.	.	.	A	.	.	T	.			
C. r. glacialis Bartlett Cove	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	C	.	.	.	.	.	.	T	G			
C. r. glacialis Excursion Inlet	.	A	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	A	C	.	.	.	.	.	.	T	.			
C. r. dawsoni Haines	.	.	.	.	.	.	.	.	C	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.			
C. r. dawsoni Canyon Island	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.			
C. r. dawsoni Turner Lake	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.			
C. r. dawsoni Crescent Lake	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.			
C. r. dawsoni Thomas Bay	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.			
C. r. dawsoni Patterson River	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	.	.	C	.	.	.	.	.	.	.	.			
C. r. dawsoni Patterson River	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.			
C. r. dawsoni British Columbia	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.			
C. r. dawsoni Yukon Territory	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	A	G	.	.	.	A	.	.			

Table 4. Uncorrected pair-wise distances (p) for *Clethrionomys gapperi*. Only unique haplotypes are shown.

		1	2	3	4	5	6	7	8	9	10
1	*C. g. stikinensis* Tyee										
2	*C. g. stikinensis* Duck Point	0.0034									
3	*C. g. stikinensis* Eagle Bay	0.0026	0.0061								
4	*C. g. stikinensis* Hoya Creek	0.0017	0.0017	0.0043							
5	*C. g. stikinensis* Frosty Bay	0.0017	0.0052	0.0043	0.0035						
6	*C. g. stikinensis* Bailey Bay	0.0026	0.0026	0.0052	0.0008	0.0043					
7	*C. g. stikinensis* Reflection Lk.	0.0034	0.0034	0.0061	0.0017	0.0052	0.0008				
8	*C. g. wrangeli* Wrangell Is.	0.0043	0.0078	0.0052	0.0061	0.0061	0.0069	0.0078			
9	*C. g. wrangeli* Wrangell Is.	0.0034	0.0069	0.0043	0.0052	0.0052	0.0061	0.0069	0.0008		
10	*C. g. wrangeli* Etolin Is.	0.0043	0.0078	0.0052	0.0061	0.0061	0.0069	0.0078	0.0000	0.0008	
11	*C. g. solus* Revillagigedo Is.	0.0034	0.0069	0.0043	0.0052	0.0052	0.0061	0.0069	0.0026	0.0034	0.0026
12	*C. g. solus* Revillagigedo Is.	0.0052	0.0087	0.0061	0.0069	0.0069	0.0078	0.0087	0.0043	0.0052	0.0043
13	*C. g. solus* Revillagigedo Is.	0.0017	0.0017	0.0052	0.0026	0.0034	0.0034	0.0043	0.0052	0.0024	0.0014
14	*C. g. solus* Revillagigedo Is.	0.0026	0.0061	0.0034	0.0043	0.0043	0.0052	0.0062	0.0034	0.0026	0.0034

Table 4. Uncorrected pair-wise distances (p) for *Clethrionomys gapperi*. Only unique haplotypes are shown (continued).

		1	2	3	4	5	6	7	8	9	10
15	*C. g. solus* Revillagigedo Is.	0.0026	0.0061	0.0034	0.0043	0.0043	0.0052	0.0061	0.0017	0.0026	0.0017
16	*C. gapperi* Bell Is.	0.0026	0.0026	0.0052	0.0008	0.0043	0.0017	0.0026	0.0069	0.0061	0.0069
17	*C. g. phaeus* Hut Point	0.0052	0.0087	0.0061	0.0069	0.0069	0.0078	0.0087	0.0061	0.0069	0.0061
18	*C. g. phaeus* Ledge Point	0.0052	0.0087	0.0061	0.0069	0.0069	0.0078	0.0087	0.0061	0.0069	0.0061
19	*C. g. phaeus* Ledge Point	0.0034	0.0069	0.0043	0.0052	0.0052	0.0061	0.0069	0.0043	0.0052	0.0043
20	*C. g. phaeus* Foggy Bay	0.0043	0.0078	0.0052	0.0061	0.0043	0.0069	0.0078	0.0052	0.0043	0.0052
21	*C. g. phaeus* Hyder	0.0026	0.0061	0.0034	0.0043	0.0043	0.0052	0.0061	0.0034	0.0026	0.0034
22	*C. g. carolinensis* N. C.	0.0603	0.0621	0.0612	0.0621	0.0621	0.0629	0.0638	0.0577	0.0586	0.0577
23	*C. g. carolinensis* N. C.	0.0612	0.0629	0.0629	0.0629	0.0621	0.0638	0.0647	0.0586	0.0594	0.0586
24	*C. g. loringi* Minnesota	0.0139	0.0174	0.0148	0.0157	0.0157	0.0166	0.0174	0.0131	0.0139	0.0131
25	*C. g. gapperi* Pennsylvania	0.0548	0.0561	0.0548	0.0573	0.0561	0.0585	0.0597	0.0535	0.0548	0.0535
26	*C. g. californicus* Washington	0.0437	0.0454	0.0446	0.0454	0.0454	0.0463	0.0472	0.0428	0.0437	0.0428

Table 4. Uncorrected pair-wise distances (p) for *Clethrionomys gapperi*. Only unique haplotypes are shown (continued).

		11	12	13	14	15	16	17	18	19	20
12	*C. g. solus* Revillagigedo Is.	0.0034									
13	*C. g. solus* Revillagigedo Is.	0.0017	0.0034								
14	*C. g. solus* Revillagigedo Is.	0.0026	0.0026	0.0008							
15	*C. g. solus* Revillagigedo Is.	0.0008	0.0026	0.0008	0.0017						
16	*C. gapperi* Bell Island	0.0061	0.0078	0.0043	0.0052	0.0052					
17	*C. g. phaeus* Hut Point	0.0052	0.0069	0.0057	0.0061	0.0043	0.0078				
18	*C. g. phaeus* Ledge Point	0.0052	0.0069	0.0052	0.0061	0.0043	0.0078	0.0017			
19	*C. g. phaeus* Ledge Point	0.0034	0.0054	0.0034	0.0043	0.0026	0.0061	0.0017	0.0017		
20	*C. g. phaeus* Foggy Bay	0.0043	0.0061	0.0026	0.0034	0.0034	0.0069	0.0078	0.0078	0.0061	
21	*C. g. phaeus* Hyder	0.0026	0.0043	0.0008	0.0017	0.0017	0.0052	0.0061	0.0061	0.0043	0.0034
22	*C. g. carolinensis* N. C.	0.0586	0.0603	0.0586	0.0594	0.0577	0.0629	0.0586	0.0586	0.0586	0.0612
23	*C. g. carolinensis* N. C.	0.0594	0.0612	0.0594	0.0603	0.0586	0.0638	0.0594	0.0594	0.0594	0.0621
24	*C. g. loringi* Minnesota	0.0122	0.0139	0.0122	0.0131	0.0113	0.0166	0.0139	0.0139	0.0122	0.0148
25	*C. g. gapperi* Pennsylvania	0.0535	0.0561	0.0548	0.0548	0.0535	0.0585	0.0522	0.0522	0.0560	0.0560
26	*C. g. californicus* Washington	0.0419	0.0419	0.0419	0.0428	0.0411	0.0463	0.0419	0.0419	0.0419	0.0419

Table 4. Uncorrected pair-wise distances (p) for *Clethrionomys gapperi*. Only unique haplotypes are shown (continued).

		21	22	23	24	25
22	*C. g. carolinensis* N. C.	0.0594				
23	*C. g. carolinensis* N. C.	0.0603	0.0026			
24	*C. g. loringi* Minnesota	0.0131	0.0551	0.0542		
25	*C. g. gapperi* Pennsylvania	0.0548	0.0038	0.0024	0.0534	
26	*C. g. californicus* Washington	0.0428	0.0603	0.0594	0.0402	0.0585

Table 5. Polymorphic cytochrome *b* sites in Alaskan and Canadian *Clethrionomys gapperi*.

Subspecies and Locality	Nucleotide Position																																				
	2 4	**1 1**	**1 2 5**	**2 4 3**	**3 8 4**	**4 1 4**	**4 2 8**	**4 7 7**	**4 8 6**	**4 9 2**	**5 0 1**	**6 0 6**	**6 0 8**	**6 1 8**	**6 7 6**	**7 9 0**	**7 1 5**	**8 1 6**	**8 5 2**	**8 5 7**	**8 7 0**	**8 8 5**	**8 9 2**	**9 4 8**	**9 7 1**	**9 8 9**	**1 0 1 4**	**1 0 1 7**	**1 0 2 8**	**1 0 8 7**	**1 1 3 4**						
C. g. stikinensis Tyee	T	C	T	C	T	G	C	T	A	C	A	G	C	C	C	T	T	A	C	A	C	C	G	A	T	C	C	A	T	A	C						
C. g. stikinensis Tyee	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.						
C. g. stikinensis Duck Point	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.						
C. g. stikinensis Duck Point	.	.	.	.	.	A	.	.	.	.	.	.	T	.	T	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.						
C. g. stikinensis Eagle Bay	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	C	.	.	.	C	.	.	.						
C. g. stikinensis Hoya Creek	.	.	.	.	.	.	.	.	.	.	.	.	T	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.						
C. g. stikinensis Frosty Bay	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.						
C. g. stikinensis Bailey Bay	.	.	.	.	.	.	.	.	.	.	G	.	T	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.						
C. g. stikinensis Reflection Lake	.	.	.	.	.	.	.	.	G	.	G	.	T	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.						
C. g. wrangeli Wrangell Island	C	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	A	.	C	.	.	.	.	.	.						
C. g. wrangeli Wrangell Island	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	A	.	C	.	.	.	.	.	.						
C. g. wrangeli Wrangell Island	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	A	.	C	.	.	.	.	.	.						
C. g. wrangeli Wrangell Island	C	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	A	.	C	.	.	.	.	.	.						
C. g. wrangeli Etolin Island	C	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	A	.	C	.	.	.	.	.	.						
C. g. wrangeli Etolin Island	C	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	A	.	C	.	.	.	.	.	.						
C. g. wrangeli Etolin Island	C	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	A	.	C	.	.	.	.	.	.						
C. g. wrangeli Etolin Island	C	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	A	.	C	.	.	.	.	.	.						
C. g. wrangeli Etolin Island	C	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	A	.	C	.	.	.	.	.	.						
C. g. wrangeli Etolin Island	C	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	A	.	C	.	.	.	.	.	.						

Table 5. Polymorphic cytochrome *b* sites in Alaskan and Canadian *Clethrionomys gapperi* (continued).

Subspecies and Locality	Nucleotide Position																														
	2 4	1 1	1 2 5	2 4 3	3 8 4	4 1 4	4 2 8	4 7 7	4 8 6	4 9 2	5 0 1	6 0 6	6 1 8	6 7 8	6 9 6	7 1 0	7 6 5	8 1 6	8 5 2	8 5 7	8 7 0	8 8 5	9 4 2	9 7 8	9 8 1	9 9 7	1 0 1 4	1 0 1 7	1 0 2 8	1 0 8 7	1 1 3 4
C. g. solus Revillagigedo Island	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	A	.	C	.	.	.	.	.	.
C. g. solus Revillagigedo Island	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	A	.	C	.	.	.	.	.	.
C. g. solus Revillagigedo Island	C	.	.	.	.	.	.	.	.	.	.	A	.	.	.	C	.	.	T	.	.	.	A	.	C	.	.	.	.	.	.
C. g. solus Revillagigedo Island	C	.	.	.	.	.	.	.	.	.	.	A	.	.	.	C	.	.	T	.	.	.	A	.	C	.	.	.	.	.	.
C. g. solus Revillagigedo Island	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	A	.	C	.	.	.	.	.	.
C. g. solus Revillagigedo Island	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	C	.	.	.	.	.	.
C. g. solus Revillagigedo Island	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	A	.	C	.	.	.	.	.	.
C. g. solus Revillagigedo Island	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	C	.	.	.	.	.	.
C. gapperi Bell Island	.	.	.	.	.	.	G	.	.	.	.	.	T	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C. g. phaeus Hut Point	C	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	T	.	.	G	A
C. g. phaeus Hut Point	C	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	T	.	.	G	A
C. g. phaeus Ledge Point	C	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	G	.	.	.	G	A
C. g. phaeus Ledge Point	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	G	A
C. g. phaeus Nooya Lake	C	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	G	A
C. g. phaeus north Rudyerd Bay	C	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	G	A
C. g. phaeus north Rudyerd Bay	C	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	G	A
C. g. phaeus south Rudyerd Bay	C	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	G	A
C. g. phaeus south Rudyerd Bay	C	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	G	A
C. g. phaeus north Smeaton Bay	C	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	G	A
C. g. phaeus south Smeaton Bay	C	.	.	T	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	A

Table 5. Polymorphic cytochrome *b* sites in Alaskan and Canadian *Clethrionomys gapperi* (continued).

Subspecies and Locality	**Nucleotide Position**																														
	2 4	**1 1**	**1 2 5**	**2 4 3**	**3 4 4**	**4 1 4**	**4 2 8**	**4 7 7**	**4 8 6**	**4 9 2**	**5 0 1**	**6 0 6**	**6 1 8**	**6 7 8**	**6 9 6**	**7 1 0**	**7 6 5**	**8 1 6**	**8 5 2**	**8 5 7**	**8 7 0**	**8 8 5**	**9 4 2**	**9 7 8**	**9 8 1**	**9 9 7**	**1 0 1 4**	**1 0 1 7**	**1 0 2 8**	**1 0 7 8**	**1 1 3 4**
C. g. phaeus Foggy Bay	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	T	A	G	C	.	.	.	.	.	.
C. g. phaeus Gwent Cove	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	T	A	G	C	.	.	.	.	.	.
C. g. phaeus Gwent Cove	.	.	C	.	.	.	.	.	.	T	.	.	.	T	.	.	.	.	.	.	.	T	A	G	C	.	.	.	.	.	.
C. g. phaeus Hyder	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	A	.	C	.	.	.	.	.	.	.
C. gapperi Salmon River, B. C.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.
C. gapperi Salmon River, B. C.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.

X. CONCLUSIONS

Using three independent approaches, morphology of the post palatal bridge, sequence variation of the nuclear intron MYH2, and RFLP analysis of the cytochrome *b* gene, Southeast Alaska distributions of *C. rutilus* and *C. gapperi* were refined. Results indicated that LeConte Bay is the primary physiographic feature separating the two species, where the southern distribution of *C. rutilus* and northern distribution of *C. gapperi* come into contact. However, evidence of unidirectional introgression was observed, whereupon cytochrome *b* haplotypes characteristic of *C. rutilus* were found in *C. gapperi* individuals up to 80 km south of the bay. Introgression is hypothesized to result from post-glacial colonization, and subsequent contact of these two species in this region after the retreat of the Laurentide and Cordilleran ice sheets.

Analysis of sequence variation of the cytochrome *b* gene provided an independent test of the five endemic subspecies red-backed voles of Southeast Alaska (*C. r. glacialis*, *C. g. phaeus*, *C. g. solus*, *C. g. stikinensis*, and *C. g. wrangeli*). The northern red-backed vole (*C. rutilus*) from Alaska exhibited low levels of sequence divergence from conspecific Old World individuals, and lacked geographic association with sequence variation. This low level of sequence divergence is hypothesized to be a result of their recent trans-Beringian colonization of North America, which corroborates evidence in the fossil record (Gromov and Polyakov, 1977). However, in the southern red-backed vole (*C. gapperi*), sequence divergence was associated with geographic location. Low but consistent levels of sequence divergence defined endemic populations in Southeast Alaska. Island populations such as Wrangell Island, and populations in

complex landscape areas, such as Misty Fjords National Monument, possessed reduced genetic diversity when compared to individuals on the mainland.

LITERATURE CITED

Gromov, I. M., and I. Y. Polyakov. 1977. Voles (Microtinae), Fauna of the USSR, Mammals, Volume II, No. 8. Smithsonian Institution Libraries and the National Science Foundation, Washington, D. C.

BIOSCIENCES LIBRARY
UNIVERSITY OF ALASKA FAIRBANKS